CHARACTERIZING EARLY DEVELOPMENT AND NREM SLEEP IN INFANTS AND TODDLERS AND RISK FOR AUTISM SPECTRUM DISORDER

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Education (Applied Developmental Sciences and Special Education)

Chapel Hill
2018

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ABSTRACT

Jessica Marie Page: Characterizing Early Development and NREM Sleep in Infants and Toddlers and Risk for Autism Spectrum Disorder
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Early childhood is characterized by rapid development and pronounced changes in early brain function and learning. Both are thought to be fostered by non-rapid eye movement (NREM) sleep. NREM sleep is characterized by the presence of slow wave activity (SWA) and sleep spindles (10-16 Hz). Both slow waves and sleep spindles exhibit pronounced developmental trajectories and are related to intelligence and general learning traits in school aged children, adults and may be altered in several neurodevelopmental disorders, such as autism spectrum disorder (ASD). Yet, these features are poorly understood in typically developing (TD) infants/toddlers and those at-risk for ASD. Moreover, it is unclear how these features are associated with outcomes of early development in TD infants/toddlers and those at-risk for ASD. This study aimed to address the following: (1) identify features of NREM sleep in 12-30-month-olds; (2) identify patterns of NREM sleep that are associated with infant/toddler development; (3) identify NREM features differentiating TD and ASD; and (4) examine NREM patterns associated with development in ASD. Using data from the Early Development and Sleep study, data collected during the home visit (measures administered: ADOS-2, MSEL, and VABS) was correlated with high density electroencephalography (hdEEG) nap recordings. The findings illustrated age related changes in delta (.5-2 Hz), theta (4-7 Hz), sleep spindles (10-16 Hz), and

beta (20-25 Hz) oscillations in the 12-30-month age span. These frequencies correlated with domain and composite scores on the MSEL and VABS. NREM findings at both the group and individual level, showed significant differences between infants/toddlers with ASD and TD. These differences were decreased theta (4-7 Hz), decreased spindles (10-16 Hz), and excessive beta (20-25 Hz) in infants/toddlers with ASD. These features were negatively correlated with performance on the MSEL and VABS and highly associated with ASD symptom severity. These findings suggest an important role of NREM sleep and the associated development of cognitive behavioral skillsets during this important developmental period. These findings provide support for the role of NREM sleep as a potential risk marker for ASD. Yet, more research is needed to further understand the application of a risk marker for research and clinical practice.

ACKNOWLEDGEMENTS

This has been the most humbling experience of my life. I am honored to have worked with world-renowned researchers and teachers in some of the most innovative and influential fields of our time. I am forever grateful to those who have helped me get where I am today and thank the following:

Mr. Pickles. You were my ride or die friend. I can only wish that everyone is fortunate to have a Mr. Pickles.

To my Mom. I watched you survive, so that one day I could thrive. Thank you for always being supportive of my dreams and goals.

To my Grandmother. You were always there for me with unconditional love and support. Till this day, you are the one person I am most afraid to let down and because of you I had a happy childhood.

MM. I am grateful to have you in my life. You have always been supportive of me and my future endeavors. Anyone who loves my cats as much as I do is a true companion.

To Kate Gallagher, my colleagues, and the teachers at the former childcare center at Frank Porter Graham (FPG) Child Development Institute. You are the reason why early educators should be acknowledged as more than teachers. You are some of the hardest working, underpaid, underappreciated professionals, and yet, provide unconditional care.

To Caroline, Sankar, and the Frohlich Lab. To Caroline, I wouldn't be here if it weren't for your mentorship and friendship. You make me want to be a better scientist. To Sankar, the "consigliere" you have been an integral part of the Frohlich lab and I cannot thank you enough

for your leadership. To everyone in the Frohlich Lab, you are some of the smartest and most innovative people I know. You have made the Frohlich Lab, “A productive, collaborative, and happy workplace. Together we will change the world!”

To my dissertation committee. Harriet, you have watched me grow as a student, teacher, and researcher. You taught me to be the biggest advocate for myself and the families we work with. I am most thankful for the opportunities you have given me and your unconditional support throughout both my Master’s and my PhD. Flavio, I am most grateful for your mentorship and support. You let me do the research that I wanted to do and stood behind me when others repeatedly told me that it could not be done. Betsy, I am thankful for your leadership and guidance. You have always made time for me and for that I cannot thank you enough. Sam, you made one of the hardest decisions that greatly impacted many lives, including mine. You have taught me the realities of providing quality care and the unconditional need for supporting families with developmental disabilities. Although this was a tough lesson, I am now a better advocate for both families and teachers and for that, I thank you. Peter, I cherish every interaction that we ever had. Your Socratic smile and constant scaffolding allowed me to question others and become an inquisitive and skeptical researcher. I thank, both you and Steve Reznick, for sharing your love and wisdom for developmental research.

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LIST OF ABBREVIATIONS AND SYMBOLS

α	Alpha
β	Beta
δ	Delta
γ	Gamma
Σ	Sigma
θ	Theta
AASM	American Academy of Sleep Medicine
ADHD	attention deficit hyperactivity disorder
ADOS-2	autism diagnostic observation schedule- 2nd edition
ASD	autism spectrum disorder
CDSA	Child Development Services Association
DL	daily living
EEG	electroencephalogram
EI	early intervention
EOG	electrooculogram
EDS	Early Development and Sleep
EMG	electromyogram
EL	expressive language
ES	effect size
FFT	Fast Fourier Transform
FM	fine motor
fMRI	functional magnetic resonance imaging
FYI	First Year Inventory
GM	gross motor
HFA	high functioning autism
hdEEG	high density electroencephalogram
IDEA	Individuals with Disabilities Education Act

M-CHAT-R	Modified Checklist for Autism in Toddlers-Revised
Mdn	median
MEG	magnetoencephalography
MSEL	Mullen Early Scales of Learning
NREM	non-rapid eye movement
PREP	pre-processing pipeline
REM	rapid eye movement
RL	receptive language
SO	slow oscillations
SOC	social domain
SnPM	statistical nonparametric mapping
SWA	slow wave activity
SWS	slow wave sleep
TD	typically developing
VABS	Vineland Adaptive Behavior Scale- 2nd edition
VP	visual perception

CHAPTER ONE: STATEMENT OF THE PROBLEM

The beginning years of life are thought to be the most sensitive periods for postnatal brain development (Gilmore et al., 2012; Shonkoff, 2000). Within this critical period is the transition from infancy to toddlerhood, in which children exhibit vast change, both at the behavioral (Levin, Zeanah, Fox, & Nelson, 2014) and neural levels (Deoni, Dean, Remer, Dirks, & O'Muircheartaigh, 2015). During this salient time period, the brain undergoes rapid alteration in neural plasticity, diverging into various trajectories, setting the foundation for later development (Shonkoff, 2000). Given this immense variation at such a young age, research is needed to better understand processes underlying development and, moreover, to pinpoint when and if early development diverges.

Considerable change is clearly observed in both early brain function and learning, and both are fostered by sleep (Kurth, Achermann, Rusterholz, & Lebourgeois, 2013), with plastic changes mirrored in the underlying architecture of the brain. Sleep is rather germane for infants as most spend the majority of their day sleeping, and as infants' age, they spend less of their day sleeping and more time engaged within their environment (Sankupellay et al., 2011). Sleep is composed of two alternating states, characterized by different eye movements, rapid eye movement (REM) and non-rapid eye movement (NREM) (Keenan & Hirshkowitz, 2011). Both REM and NREM promote cortical development, the establishment of connectivity, and are vital to processes underlying cognitive and behavioral functioning (Deoni et al., 2015) and yet, the function of both REM and NREM are not fully understood (Bathory & Tomopoulos, 2017).

The development of sleep and features of NREM sleep are believed to be an indicator of maturation of the central nervous system (CNS) (Shinomiya, Nagata, Takahashi, & Masumura, 1999). Cortical maturation is reflected in changes of the sleep electroencephalogram, EEG (Huber & Born, 2014; Novelli et al., 2016), specifically within NREM sleep (Kurth, 2010; Lee, Fattinger, Mouthon, Noirhomme, & Huber, 2013; Novelli et al., 2016; Sankupellay et al., 2011). Dominant brain oscillations during NREM sleep, such as sleep spindles and slow waves, are associated with cognitive functioning (Doucette, Kurth, Chevalier, Munakata, & LeBourgeois, 2015; Tessier & et al., 2015), learning efficiency (Lustenberger, Maric, Durr, Achermann, & Huber, 2012), memory consolidation (Lustenberger, Wehrle, Tushaus, Achermann, & Huber, 2015; Rihm, Diekelmann, Born, & Rasch, 2014), and motor skill development (Kurth et al., 2013; Lustenberger et al., 2017). Although the network dynamics (activity patterns) of sleep are thought to reflect connectivity and overall brain organization, it is unclear what features of NREM sleep moderate plasticity in early development. Moreover, elucidating what factors of NREM moderate typical from atypical development in neurodevelopmental disorders, such as Autism Spectrum Disorder (ASD), is also unknown. Demarcating normative features of sleep in young children represents an important foundation for understanding brain maturation and may ultimately serve as a marker of brain development.

ASD is a heterogeneous neurodevelopmental disorder with variations exemplified in a child's intellectual abilities, language proficiency, and age (Tager-Flusberg & Joseph, 2003). Individuals with ASD show impairments in social communication and restricted/repetitive behaviors (DSM-5; American Psychiatric Association, 2013) with high familial risk, upwards to 20% (Ozonoff et al., 2011). Though 1 in 59 is diagnosed with ASD (ADDM, 2018), diagnosis in very young infants is challenging as it relies on behavioral measures that depend on the child's

motivation, professional clinical judgment, and presence of symptomatology (Liptak et al., 2008) that becomes more pronounced during the second year of life (Barton, Dumont-Mathieu, & Fein, 2012; Filipek et al., 1999; Ventola et al., 2007). Despite the onset of behavioral symptoms beginning as young as 6 months (Ozonoff et al., 2010; Ozonoff et al., 2011) and underlying neural differences observed at 6 months (Tierney, Gabard-Durnam, Vogel-Farley, Tager-Flusberg, & Nelson, 2012), the average age of diagnosis is 4 years (ADDM, 2018). .

Infants and toddlers identified with a significant delay or developmental disability before 36 months of age are eligible for Part C, early intervention (EI) services. An early diagnosis affords access to EI, leading to improved developmental outcomes (Dawson et al., 2012). When children are diagnosed later, they are not likely to receive EI and, thus, don't benefit from EI services. An objective measure, such as a biological marker, allows for a risk marker and identification of a young child at-risk of ASD well before the onset of behavioral symptoms. If a biomarker is found to predict core features (symptoms), this could justify access to EI and allow research to further examine how EI alters symptoms at both the neurological and behavioral level (Dawson et al., 2012).

It is unclear what features in the brain underlie the development of ASD and, thus, research has yet to identify a biological risk marker of ASD. Sleep is thought to underlie development and can be studied to elucidate how the brain develops and further our understanding of the onset of ASD. The remainder of this chapter provides an overview of the relevant research on early development and sleep in infants and toddlers that are both typically developing (TD) and at-risk for ASD. Finally, the aims of this study and research questions are provided.

Development of NREM Sleep from Infancy to Toddlerhood

Across development, sleep is widely investigated using EEG or high density electroencephalography (hdEEG) in neonates (Chu, Leahy, Pathmanathan, Kramer, & Cash, 2014), young children (e.g. 2-19 years, Kurth et al., 2013) and throughout adulthood (De Gennaro, Ferrara, Vecchio, Curcio, & Bertini, 2005), showing clear maturational differences in both state- and frequency-dependent changes (Jenni, Borbely, & Achermann, 2004). As infants age, there is a general decrease in REM and an increase of NREM sleep (Jenni et al., 2004). Maturation is further depicted in NREM sleep, with observed changes in low frequencies becoming increasingly dense (Sankupellay et al., 2011) and an increase in higher frequencies (Chu et al., 2014). Dominant oscillations during NREM sleep are sleep spindles (10-16Hz) and slow waves (< 5 Hz), and both have been associated with behavioral performance and general traits of learning (Lustenberger et al., 2012).

Sleep Spindles

One feature that is highly associated with maturation during NREM are sleep spindles. Sleep spindles are thalamo-cortically generated brain rhythms at 10-16 Hz (De Gennaro et al., 2005) and are associated with intelligence and processing speed in preschool-aged children (Doucette et al., 2015). Sleep spindles are detected in infants at four to nine weeks of age and (Ellingson, 1982) are widely studied in older children (2 – 15 years) into adulthood. Sleep spindles undergo change in length and density throughout development (Kurth, 2010; McClain et al., 2016; Scholle, Zwacka, & Scholle, 2007) and are altered in several disorders, including ASD (Tessier et al., 2015). Sleep spindles are delineated into slow, medium, fast, and more recently "ultrafast" (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017) though most commonly described as being slow or fast. Variations of a sleep spindle are found in single or slow spindle (11-12.75 Hz) in frontal regions to a double or fast spindle (12.5-16 Hz) in centroparietal regions

(Shinomiya et al., 1999). Both slow and fast spindles show maturational differences in which fast spindles increase with age, compared to slower spindles that simultaneously decrease and increase with age (Shinomiya et al., 1999), further depicting anatomical and physiological properties of the thalamocortical system (Lüthi, 2014). Dynamics of the thalamocortical reticular nucleus are the source of sleep spindles (De Gennaro & Ferrara, 2003), and deviations within the thalamocortical system may provide further insight into typical and atypical development.

Slow Wave Activity (SWA)

Within NREM sleep are topographical alterations of delta band slow wave activity (SWA) between 0.5 and 4.0 Hz. Slow waves are composed of down states (hyperpolarization) in cortical and thalamocortical neurons (Massimini, Tononi, & Huber, 2009) and are believed to serve as a restorative function during sleep (Borb & Achermann, 1999; Jenni et al., 2004). The amplitude of SWA serves as a marker of sleep pressure; and, throughout the course of the night, SWA increases as a function of prior waking and decreases throughout sleep (Borbély, 1982). Slow waves exhibit a maturational shift from posterior to frontal regions; and, within infants and young children, there is increased occipital SWA which is maximal in the occipital cortex (Fattinger, Jenni, Schmitt, Achermann, & Huber, 2014), and greater power in theta and sigma frequencies (Kurth, 2010; Novelli et al., 2016). Local features of SWA are thought to reflect plastic changes prompted by general traits of learning (Bódizs, Gombos, Ujma, & Kovács, 2014; Huber, Ghilardi, Massimini, & Tononi, 2004) such as memory (De Gennaro & Ferrara, 2003) and processing speed (Doucette et al., 2015). Collectively, research examining SWA and sleep spindle characteristics find stark differences across typical control populations versus populations with neurodevelopmental disorders, such as ASD (Limoges et al., 2005).

Sleep in ASD Populations

Neurodevelopmental disorders such as ASD are thought to arise from disrupted circuits of the CNS (Singh, Lin, Newell, & Nelson, 2002; Wang et al., 2013). One area of the brain that is thought to be implicated in populations with ASD is the temporo-occipital region, spanning to thalamo-cortical areas underlying information processing, sleep, and core features of ASD (Daoust, Limoges, Bolduc, Mottron, & Godbout, 2004). To date, only a few studies have examined EEG during sleep with young children (Buckley et al., 2015; S. Tessier, Lambert, Scherzer, Jemel, & Godbout, 2015) or adults with ASD (Limoges, Mottron, Bolduc, Berthiaume, & Godbout, 2005) which is surprising considering 50 - 80% of parents report sleep difficulties in children with ASD (Doo & Wing, 2006; Polimeni, Richdale, & Francis, 2005; Richdale & Schreck, 2009). Only a handful of studies have examined features of NREM sleep (sleep spindles and SWA) in populations with ASD, and no study has investigated the two prominent features of NREM sleep characteristics in infants and toddlers with or at-risk for ASD.

Populations with ASD exhibit longer sleep latency, more frequent nocturnal awakenings, lower sleep efficiency (Limoges et al., 2005), and increased prevalence of sleep disorders (Malow et al., 2006b; Taylor, Schreck, & Mulick, 2012). In other words, it takes longer to fall asleep, with increased duration of stage 1 sleep (light sleep), decreased NREM sleep (deep sleep), fewer sleep spindles, and fewer periods of REM sleep (Léveillé et al., 2010; Limoges et al., 2005; Tessier et al., 2015). These findings indicate that many features of sleep are atypical in populations with ASD and consistent with other research that suggests neural networks are altered in populations with ASD (Wang et al., 2013), resulting in the core symptoms of ASD (Luckhardt, Jarczok, & Bender, 2014; Ruggeri, Sarkans, Schumann, & Persico, 2014).

Sleep is understudied in infant/toddlers and specifically those with or at-risk of ASD and thus it is unclear if and what features of NREM sleep are altered in this population. Identifying

features of NREM sleep that are altered in infants/toddlers at-risk of ASD further suggests a risk biomarker of ASD. With a biomarker in place this could suggest increased risk of ASD well before the presence of behavioral symptoms.

Aims of the Current Study

Sleep has yet to be fully examined in infants and is relatively understudied during the important transition from infancy into toddlerhood. Moreover, very little is known about sleep in infants and toddlers at-risk of ASD. Given the maturational features of sleep, which are believed to parallel development, and the limited knowledge of sleep in both typically developing and at-risk infants and toddlers, sleep may be a possible marker of ASD. To date, no studies have used hdEEG during sleep or daytime naps in infants or toddlers with or at-risk for ASD for the purposes of identifying a biomarker. As such, it is unclear how sleep develops across infancy into toddlerhood and the potential to ascertain sleep as a candidate risk marker of ASD. Given the two major gaps in the research base on sleep in the transition period to toddlerhood and sleep in populations with ASD, this study will address the following research questions:

Research question 1a. What are the physiological activity patterns of NREM sleep (sleep spindles and SWA) in infants/toddlers 12-30 months of age?

Research question 1b. What physiological activity patterns of NREM sleep are associated with development in infants/toddlers 12-30 months of age?

Research question 2a. Given the physiological activity patterns of NREM sleep in infants/toddlers, what patterns of NREM sleep are associated with ASD?

Research question 2b. Given the physiological activity patterns of NREM sleep and associations with development in infants/toddlers, what features of NREM sleep are associated with development in infants/toddlers with ASD?

CHAPTER TWO: REVIEW OF THE LITERATURE

Early childhood is marked by vast developmental change reflected at both the behavioral and neural level. Within this time period is the transition from infancy to toddlerhood. Traditionally, infant and toddler development is assessed at the behavioral level with a variety of assessments during the wake state. Yet, much of what is learned and experienced throughout the day is processed and restored during sleep (Tononi & Cirelli, 2006). As such, studying sleep may serve to better understand the interplay between development, learning, and the underlying neural networks during this important time period.

Although sleep has a universal presence (found in all animals, (Cirelli & Tononi, 2008)) and humans spend a third of their life asleep (Cirelli & Tononi, 2004), the core neural features of sleep and the influence on development are poorly understood. There is some agreement that the primary function of sleep is a state where synaptic plasticity is regulated (Cirelli & Tononi, 2004) acting as a highly organized, structured, and restorative feature (De Gennaro & Ferrara, 2003; Tononi & Cirelli, 2006). Alterations in the sleep network dynamics (neural level) reflect development of the CNS (Andrillon et al., 2011; Tanguay, Ornitz, Kaplan, & Bozzo, 1975), and this is mirrored in maturational, regional (local), and global change of the sleep topography (spread or variation of electrical activity) (Kurth, 2010).

Across development, the sleep topography undergoes massive change, which is exhibited in the sleep characteristics during rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. Specifically, features of NREM sleep are shown to be highly associated with enhanced learning in preschool children (Doucette et al., 2015; Kurdziel, Duclos, & Spencer,

2013), cognitive abilities in older children (Chatburn et al., 2013) and memory in adults (Lustenberger et al., 2012). Despite these findings in older populations, it is unclear if and what features of sleep are associated with cognitive and behavioral development in infancy and toddlerhood. Characterizing maturational and regional changes during NREM sleep is an important step to understand development and learning, furthermore, providing a baseline to examine associations of typical development. Data of NREM sleep with typically developing infants/toddlers would provide a baseline measure and may further elucidate when and what disruptions in cortical development arise in neurodevelopmental disorders, such as autism spectrum disorder (ASD).

Prior research suggests that the sleep network dynamics are altered in older populations with ASD (Buckley et al., 2015; Godbout, Bergeron, Limoges, Stip, & Mottron, 2000), and the extent to which there are affected sleep networks in populations that are at-risk of ASD (infant sibling of ASD) is unknown. With a baseline measure in place, this may further pinpoint when and what features of development diverge in infants at-risk of ASD, thus serving as candidate risk marker(s) for ASD. A risk marker of ASD could offer an opportunity for intervention before the onset of behavioral symptoms, and, with an intervention in place, may lead to decreased symptomology and increased behavioral outcomes (Dawson et al., 2012).

This chapter begins with a discussion of the maturation of cortical activity in NREM sleep. Alterations in cortical activity in NREM have been linked to neurodevelopmental disorders such as schizophrenia (Gardner, Kersanté, Jones, & Bartsch, 2014; Lustenberger et al., 2015). These findings further highlight research showing disruptions in cortical activity in populations with ASD (Wang et al., 2013), thus likely affecting development. One dominant theory suggests that individuals with ASD have aberrant cortical activity (David et al., 2016;

Polleux & Lauder, 2004; Wang et al., 2013). Thus, examining cortical activity in the sleep state in both TD infants/toddlers and infants/toddlers with or at-risk of ASD is an important step to further detect if and what disruptions of cortical activity arise in NREM sleep in the infant/toddler years.

Traditionally, sleep is measured with electroencephalography (EEG), to quantify sleep into the primary sleep stages: rapid eye movement (REM) and non-rapid eye movement (NREM). Two differentiating features, sleep spindles and slow wave activity (SWA), are present during NREM sleep. SWA and, specifically, sleep spindles are both highly associated with cognition and general traits of learning in young children (Doucette et al., 2015), adolescents (Astill et al., 2014; Chatburn et al., 2013) and memory in adults (Fogel, Nader, Cote, & Smith, 2007). Therefore, an in-depth discussion of the development of these features from birth to early childhood and the associated features of sleep spindles and SWA follow.

Although a body of research examines sleep across the lifespan, research measuring sleep with EEG in populations with ASD is rather limited. Only a handful of studies examine features of NREM sleep (sleep spindles and SWA) in populations with ASD, with the bulk of research showing altered sleep spindle characteristics in older children and adults (Limoges et al., 2005). Thus, a brief examination of sleep spindles and SWA in populations with ASD and their associations with behavior and features of ASD are put forth. Despite pronounced changes of sleep spindles (McClain et al., 2016) and SWA (Kurth, 2010) and their associated traits across the lifespan, these prominent features of NREM sleep are poorly understood in infants and toddlers in the age range of 12 to 30 months (Novelli et al., 2016) and are largely ignored in the literature on ASD. This gap in our knowledge base is addressed here, with a detailed explanation of the proposed study's aims and research questions.

Cortical Activity: Maturation in Early Childhood

An attribute of early development is cortical maturation, and specifically, experience-dependent plasticity (Wilhelm et al., 2014). Cortical plasticity reflects the variation in the number of synapses or the flexibility of synaptic connections that can be altered (Kurth et al., 2016; Schumacher et al., 2017) and are adaptive to change (Wilhelm et al., 2014). Cortical plasticity is most prominent during early childhood (Ringli & Huber, 2011), when the brain undergoes periods of sensitive and critical development (Wilhelm et al., 2014). Here, sensitive means that the brain is acutely responsive to experiences mirrored in patterns of cortical activity (Cynader & Mitchell, 1980; Fox, Levitt, & Nelson, 2010) or in which the presence or absence of an experience results in a lasting or permanent state (Fox et al., 2010; Newport, Bavelier, & Neville, 2001). Additionally, cortical development is altered by exposure or lack of exposure to various environmental, maturational, and genetic traits, further changing development in various ways (Kurth et al., 2016). Given a variety of influences during critical and sensitive periods, sleep may be important for processes associated with neurodevelopment (Kurth et al., 2016). It is important to understand the factors that deter typical development, as well as influences that support optimal development in infancy and toddlerhood when the brain is most plastic. Moreover, understanding the role of sleep and its influence on plastic processes and the potential to moderate neurodevelopment is of great importance during this time period.

Research using MRI shows that cortical pathways processing lower-level information mature much earlier than those processing higher level information (Fox et al., 2010; Scherf, Behrmann, Humphreys, & Luna, 2007). These maturing paths depict local and global changes that are correlated with cognitive ability and intelligence (Deoni et al., 2015; Stickgold, 2005) and correlated with neurodevelopmental disorders (Limoges et al., 2005; Shaw et al., 2007;

Shaw et al., 2008). Other non-invasive measures with high temporal resolution such as EEG, also show maturation of cortical activity during the sleep state. In NREM sleep, from infancy to toddlerhood, age and regional changes are clearly observed, depicting a time where developmental trajectories diverge (Chu et al., 2014) and, likely, are more sensitive to adaptation. For example, at 2 months, there is a prominent increase in frontocentral regions of the power in delta (.05-4 Hz), theta (4-7 Hz), and sigma (10-16Hz) (Chu et al., 2014; Sankupellay et al., 2011). From 6 to 36 months, there is a shift in frontal and central regions, with noticeable increases in higher frequencies of beta (16.25-20) and gamma (30-40Hz) (Chu et al., 2014). The increase in power from .05-40 Hz (Chu et al., 2014) along with features of NREM, sleep spindles and SWA (Kurth, 2010), parallel the progression of cortical maturation (Ringli & Huber, 2011). Thus, given the relatively stable increase in power and change of both sleep spindles and SWA, these attributes may depict a timeline of what cortical activity becomes altered in populations that are at-risk for ASD.

Altered Cortical Activity and Risk of ASD

Research with functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), and, specifically, electroencephalography (EEG) consistently detects disruptions at the cortical level, during both wake and sleep in populations with ASD. Furthermore, alterations in sleep spindle characteristics and SWA are highly associated with behavior and implicated in patients with neurodevelopmental disorders such as schizophrenia (Ferrarelli et al., 2007; Lustenberger, O'Gorman, et al., 2015), attention deficit hyperactivity disorder (ADHD) (Arns & Kenemans, 2014; Ringli et al., 2013), depression (Sesso et al., 2017), and ASD (Godbout et al, 2005; Limoges et al, 2005; Tessier et al, 2015). One dominant theory of ASD suggests that individuals with ASD have disrupted cortical activity, posing alterations in neural plasticity,

triggering atypical synchrony or coherence among neuronal populations (Ghuman, van den Honert, Huppert, Wallace, & Martin, 2017), abnormalities in neural connections (Adolphs, Sears, & Piven, 2001; Buckley et al., 2015; Righi, Tierney, Tager-Flusberg, & Nelson, 2014), differences in oscillatory power (Tierney & Nelson, 2009), and atypical spindle characteristics (Tessier et al., 2015). Disruptions of cortical activity in any of these components is thought to give rise to the observed symptoms and behavioral differences in social communication and restricted and repetitive behaviors (core features of ASD).

Despite the body of research using EEG and findings of altered cortical activity in populations with and at-risk of ASD, there are many inconsistencies and differences across research studies. For example, there are differences in cortical activity at various frequencies and bandwidths (Wang et al., 2013). Thus, it is unclear which differences are frequency-band specific, the differences in power (Maxwell et al., 2015) and asymmetry (Gabard-Durnam, Tierney, Vogel-Farley, Tager-Flusberg, & Nelson, 2015; Tierney & Nelson, 2009) that occur, in the wake state, and differences due to the specific task(s) (Banaschewski & Brandeis, 2007). Although there is wide heterogeneity in populations with ASD, with various disruptions at the neural level, sleep provides an avenue to address some of the ambiguities that arise. However, it is unclear what and how neural activity is altered during sleep in infant/toddler populations that have or are at-risk of ASD. Examining neural activity of the sleep topography of infants with and at-risk of ASD will elucidate some of the noted inconsistencies and provide another avenue to assess the extent to which neural activity may be altered in infants/toddlers with and at-risk of ASD.

EEG and the Assessment of Sleep

At the behavioral level, we can roughly determine if one is asleep. The addition of EEG allows for a more refined quantification of sleep (Achermann, 2009; Geyer, Talathi, & Carney, 2009), permitting the classification of various sleep stages. Rechtschaffen and Kales (1968) developed the first human sleep stage scoring guidelines. These have been adapted, and today's American Academy of Sleep Medicine guidelines (AASM) (Iber et al., 2007) are the most widely used criteria for demarcating sleep stages. Sleep is typically quantified with polysomnography which consists of EEG, electrooculography (EOG, eye movement), electromyogram (EMG, muscle movement), and electrocardiogram (EKG or ECG, heart activity). Collectively, these are important measures used to differentiate and delineate sleep states (stages). Before the in-depth discussion on sleep, it is important to briefly examine the mechanisms of EEG, as this is the primary method used in sleep laboratories and in the proposed study. After a brief introduction describing the basic components of EEG and what it measures, a discussion on the use of EEG in sleep and the quantification of sleep using the AASM guidelines will follow.

Electroencephalography (EEG)

Electroencephalography (EEG) is a non-invasive measure of brain processes in real time with high temporal resolution. EEG captures rapid neural events (Banaschewski & Brandeis, 2007), reflecting mass neural activity (group of neurons) of voltages generated by excitatory postsynaptic potentials from apical dendrites of synchronized neocortical cells in the neocortex (Fröhlich, 2016; Nunez & Srinivasan, 2006; Srinivasan, Nunez, & Silberstein, 1998; Srinivasan, Winter, & Nunez, 2006). Synchronized neural activity (Nunez & Srinivasan, 2006; Srinivasan et al., 2006) at the cortical (surface) level consists of current flows which are measured as differences in electrical potential (Banaschewski & Brandeis, 2007; Fröhlich, 2016). The

temporal synchronization of neural networks may be transient, slow, or oscillatory (waveforms, Banaschewski & Brandies, 2007), and this is used to describe cortical activity in both the wake and sleep states.

EEG research can detect the processing of task-irrelevant, distracting, unattended stimuli, or no task (Achermann, 2009; Banaschewski & Brandeis, 2007), showing marked changes observed with age (Tierney et al., 2012), and can be used with challenging populations, such as infants (Banaschewski & Brandies, 2007; Fox et al., 2001;Tierney et al., 2012). Moreover, EEG provides a means to show if the underlying neural activity collected during task performance reflects the same processes that are seen at the behavioral level, which can be used to compare groups across development (Righi, Tierney, Tager-Flusberg, & Nelson 2014), as well as longitudinal differences in the same group (Tierney et al., 2012) and across disparate populations (Banaschewski & Brandies, 2007).

Measuring Sleep: Sleep Stages

Given frequency, amplitude and waveform differences in the EEG, sleep can easily be differentiated from wake and sleep states (Achermann, 2009; Iber et al., 2007). Sleep states are divided into two states, Non-rapid eye movement sleep (NREM) and rapid eye movement (REM) sleep (Iber et al., 2007; Rechtschaffen & Kales, 1968). NREM sleep can be divided further into NREM stages N1, N2, and N3 (Iber et al., 2007). Sleep stage N1 consists of the transition between wakefulness and sleep and is characterized by sleep onset, a decrease in alpha power, and an increase of low-voltage frequencies and sharp vertex waves. It is sometimes referred to as light sleep (Achermann, 2009). Stage N2 is characterized by the occurrence of sleep spindles and K-complexes, which often occur in a sequential fashion and are a hallmark of N2. Stage N3 (formally 3 and 4) is referred as slow wave sleep (SWS). During N3, low voltage

frequency waves (delta) emerge, also known as slow wave activity (SWA). Slow waves are composed of periods of hyperpolarization and depolarization (change in membrane potential moving toward more negative or positive) (Astill et al., 2014; Kurth et al., 2010) encompassing at least 20% of the EEG (Iber et al. 2007), and may occur in parallel with sleep spindles (later in the chapter is an in-depth discussion of sleep spindles and SWA).

One of the most well-known sleep states is REM sleep. Although REM is known for periods in which dreaming occurs, during REM, brain activity is desynchronized. In addition to desynchronized activity, there are bouts of electromyogram (EMG) showing atonia (lack of muscle tone or strength) (Iber et al., 2007), and the electrooculogram (EOG) consists of rapid eye movements for which REM is known. By 2-to-3-years of age, there is a significant decrease in REM (25% of the night) and an increase in NREM (Huber & Born, 2014). Over the course of an 8-hour sleep cycle, humans alternate between periods of NREM and REM sleep, lasting between 90 to 120 minutes per state (Feinberg & Floyd, 1979). Thus, the average adult undergoes 4 to 6 NREM-REM sleep cycles. Although REM sleep is shown to be altered in populations with ASD (Buckley et al., 2015; Tessier et al., 2015), the focus of the proposed study is NREM sleep, and thus, there will be no comprehensive review of REM sleep.

Sleep Scoring

Sleep scoring is used to describe the sleep architecture. Traditionally, sleep scoring and spectral analysis are performed with a minimum of 2 electrodes; however, recent studies show that EEG during sleep contains both local and global elements (Achermann, 2009; Murphy et al., 2009). One way to assess the whole head is with high density EEG (hdEEG). High density EEG is measured with 64 or more electrodes, covering most of the upper surface of the head. One advantage of hdEEG is that it allows for increased temporal resolution with increased spatial

resolution permitting global and local topographical measurement (Achermann, 2009). Another advantage is that mores electrodes allow for a more comfortable measurement. With the use of hdEEG, one can score and differentiate global versus local sleep across the two prominent sleep states, NREM and REM. Though REM sleep is the more well-known sleep state, it is during NREM sleep that our brain undergoes a restorative state (Ringli & Huber, 2014). Specifically, N2 and N3 are known for the presence of sleep spindles and SWA, respectively. The next few sections outline the components and associations of sleep spindles, and then SWA, with behavior across early childhood, which is followed by an examination of these components in populations with ASD.

Sleep Spindles: Components and Attributes

Sleep spindles are thalamocortical derived activity (De Gennaro et al., 2005; Kandel & Buzsáki, 1997; Steriade, 2006) occurring between 10-16Hz (sigma bandwidth) and consisting of a waxing and waning of the EEG lasting between 0.5-2 seconds (Gibbs & Gibbs, 1941). Sleep spindles typically occur during both light sleep (NREM N2) and the active (depolarized) phase of slow oscillations, deep sleep (NREM N3) and reoccur every 5-15 seconds (Lüthi, 2014; Timofeev, Bazhenov, Seigneur, & Sejnowski, 2012). The sleep spindle has features that are demarcated into spindle duration (time), amplitude (power) and density (number/min) (De Gennaro & Ferrara, 2003) To obtain these different measures, visual or automatic spindle detection is performed (De Gennaro & Ferrara, 2003; Ferrarelli et al., 2007) which allows for a topographical representation providing an illustration of where spindles are located. Both sleep spindle measures and their associated topographies show prominent between-participant variability and pronounced within-participant stability (De Gennaro & Ferrara, 2003). Thus, sleep spindles have trait-like aspects that are reproducible across time (Chen, Gully, Whiteman,

& Kilcullen, 2000), and are comparable to a biological fingerprint (Finelli, Achermann, & Borbély, 2001). In addition to trait-like aspects, sleep spindles also show state-like facets (variations under specific situations). For example, if one has been sleep deprived or traveled across various time zones, these instances can alter spindle properties (Lüthi, 2014).

Sleep Spindle: Characteristics in Early Childhood

Across development, numerous changes occur in spindle activity showing wide change in spectral power and spindle characteristics (Clawson, Durkin, & Aton, 2016; De Gennaro & Ferrara, 2003). Spindles are observed in infants as young as 1-2 months (Ellingson, 1982). The formation of slow spindles occur around 24 months are (10-13 Hz) and fast spindles (13-16 Hz) (Jankel & Niedermeyer, 1985). During early development, slower spindles show a more dramatic change compared to faster spindles. Slower frequencies are found over frontal regions and are more pronounced during slow wave sleep (SWS, N3), whereas faster frequencies are located over centroparietal regions and are prominent during Stage 2 sleep (N2) (Andrillon et al., 2011; Astill et al., 2014b; Doucette et al., 2015).

From infancy to adolescence, spindles generally become faster, (Clawson et al., 2016; De Gennaro & Ferrara, 2003; Jenni et al., 2004; Shinomiya et al., 1999). During early infancy, sleep spindles show asymmetry and alternate between hemispheres, though with age become more synchronous (Gruber & Wise, 2016e44). Throughout infancy, there is a gradual increase of power in sigma bandwidth (spindle band) from 12.6 Hz at 3 months, 12.8 at 6 months, and 13.3 at 12 months of age (D'Atri, Novelli, Ferrera, Bruni, & De Gennaro, 2018; Sankupellay et al., 2011). At 4-12 months, there is increased density and faster frequencies in frontal, central, and parietal regions. Yet, around 18 months, there is a maximal 11 Hz frontal peak along the mid-line in the slow spindle band (Chu et al., 2014; Novelli et al., 2016). From 24 to 60 months there

is increased duration and frequency of frontal spindles but no significant increase in spindle density (number/min) after 12 months (D'Atri, D'Atri, Novelli, Ferrera, Bruni, & De Gennaro, 2018; McClain et al., 2016; Sankupellay et al., 2011). Cross sectional data covering infancy to adolescence (Chu et al., 2014; Kurth et al. 2010) depict spindle density, and length (duration of the spindle) exhibits an inverted U-shaped profile with minimal density at 19 to 36 months (Chu et al., 2014; D'Atri, Novelli, Ferrera, Bruni, & De Gennaro, 2018) and minimal length between 19 to 27 months (Scholle et al., 2007). During this age range there are clear differences in power, duration, amplitude, length, and density of sleep spindles which are seen with development. Reasons for the observed change include the direction and amount of change in the age span, and the electrode montage (number of electrodes on the scalp), as research shows clear topographical differences (Chu et al., 2014; De Gennaro & Ferrara, 2003; Kurth et al., 2010; Shinomiya et al., 1999). Despite the wide change in such a short period of time, research in adults shows that sleep spindle density is stable from night-to-night (De Gennaro & Ferrara, 2003; Fogel et al, 2011), and sleep spindle characteristics are highly associated at the behavioral level.

Sleep Spindles: Associations with Behavioral Performance

Alterations of sleep spindle characteristics are thought to be a reflection of the maturation of the CNS (Andrillon et al., 2011; Tanguay et al., 1975), and in addition to their changing topography across development. In adults, fast spindle characteristics are positively correlated with more complex cognitive abilities as IQ, procedural memory, declarative memory, and various aspects of learning (Fogel et al., 2007; Schabus et al., 2006). Whereas slow spindles have been associated with visual perceptual learning (Gruber & Wise, 2016). Research examining relationships of sleep spindles and behavior in children is more inconsistent. One reason for the inconsistency is based upon the spindle frequency of interest (Clawson, Durkin, & Aton, 2016;

Fogel et al., 2011). Fast spindles correlate negatively with measures of IQ and perceptual reasoning (Fogel & Smith, 2011), working memory, sensorimotor, and planning (Chatburn et al., 2013) in school-aged children. Whereas slow sleep spindles show a positive correlation with measures of IQ in school-aged children (Clawson et al., 2016) and processing speed in preschool-aged children (Doucette et al. 2015). To date, no studies have examined sleep spindle characteristics and associations with IQ or any other developmental domain that is typically assessed in early development with infants and toddlers. Despite the scarcity of research with infants and toddlers, many studies have examined sleep spindle characteristics in school aged children and to a lesser extent preschool aged child.

School Age

Sleep spindles are widely associated with IQ in school aged children. Across three similar research studies, spindle frequency is the most consistent finding influencing cognitive performance. Geiger et al. (2011) examined 14 children (mean age 10.5 years) to investigate associations with all night sleep recordings and intellectual ability (WISC-IV, full scale and fluid IQ (reasoning/problem solving)) and performance with working memory and speed of processing. Findings show peak spindle frequency was negatively correlated with full scale IQ (WISC-IV) whereas spindle power showed positive correlations with fluid IQ. In a similar study, Gruber et al. (2013) examined 29 school aged children (mean age 8.79 years) to assess if spindle amplitude, density, duration, and frequency were associated with the following domains on the WISC-IV: perceptual reasoning, verbal comprehension, and working memory. Like Geiger et al. (2011), only sleep spindle frequency was negatively correlated with better performance on perceptual reasoning and working memory. Spindle amplitude, duration, and density were not associated with any measures of the WISC-IV. More recently, Hoedlmoser et al. (2014)

examined 62 children (mean age 9.56 years) in two overnight sleep visits and assessed the role of sleep spindle activity, declarative memory consolidation (number of recalled words) and cognitive performance on the WISC-IV. Children with higher sleep spindle frequencies at frontal, central, parietal, and occipital sites exhibited higher performance on both the WISC-IV and declarative memory consolidation. Thus, children with faster sleep spindles were more likely to recall more words before and after sleep.

Chatburn et al. (2013) examined 27 children (mean age 8.19 years) to assess associations of slow and fast spindle characteristics and performance on the Stanford Binet Intelligence scale and the neuropsychological development assessment (NEPSY). The NEPSY assesses a wide array of skillsets, such as memory and learning, sensorimotor function, language development, planning and problem solving. Findings showed that density (number) of fast spindles was positively associated with memory and negatively correlated with sensorimotor functioning. Similar though slightly different from other findings, average frequency of sleep spindles was negatively correlated with planning ability and working memory.

In a similar study, Astill et al. (2014) examined the correlations of sleep spindle and SWA characteristics and motor skill (finger tapping sequence) in 30 children (mean age 10.7 years) during 12 or 24-hour periods of wake and sleep. Overall, children had better performance (increased speed) if they had fewer slow spindles and more fast spindles. Accuracy increased only with the sleep condition and was most pronounced in children with more slow spindles. In other words, the children with a lower baseline performance showed the greatest improvement in accuracy which was correlated with slow spindles. Collectively, research examining sleep spindle characteristics and performance with various aspects of cognitive performance, show sleep spindle frequency and density are associated with different aspects of cognitive

performance and motor skills in school aged children. This suggests, that features of slow and fast spindles are associated with different cognitive skillsets and thus, have different roles in learning and development of various skills.

Preschool

In preschoolers, spindle characteristics are also associated with processing speed (reaction time). In a small cross-sectional study, Doucette at el 2015 examined the associations between processing speed and sigma power (slow and fast spindles). Findings showed a clear peak in slow sigma (spindles) with greater power in parietal regions that was associated with faster processing speed (faster reaction time) (Doucette et al., 2015). These findings highlight the work of Astill et al. (2014) showing a positive correlation with spindles and faster response time in a motor task. Beyond the different age ranges, one reason for the observed difference between Astill et al. (2014) and Doucette et al. (2015) is that some of the preschool children in Doucette et al. may not have developed the pronounced double spindle. Thus, some children may have only had a more prominent fast or slow spindle.

Kurdziel et al. (2013) examined 4-year-old children and the benefit of a daytime nap on a visuospatial task, similar to the game of Memory. Baseline performance showed a negative correlation of spindle density, rather children with lower baselines had increased opportunity to improve, similar to the baseline performance in Astill et al. (2014) and Chatburn et al. (2013). After the nap, sleep spindle density was positively correlated with improvements in children's visuospatial performance.

Collectively, research in school aged and preschool children, shows that both slow and fast spindle characteristics are correlated with various aspects of behavioral performance. Interestingly, there are some differences, where processing speed in preschoolers was correlated

with slow spindles (Doucette et al.). Whereas fast spindles show positive correlations with faster response (Astill et al., 2014) and memory (Chatburn et al. 2013), increased density also shows a positive correlation with visuospatial performance in a memory task (Kurdziel et al., 2013). Thus far, no studies have examined associations of cognitive performance, processing speed, or other measures of behavioral performance in children younger than 36 months of age. Furthermore, the slow and fast or "double spindle" is pronounced around 24-months of age (Jankel & Niedermeyer, 1985) as such, it is unclear if there are associations with fast spindles and cognitive performance, slow spindles and, moreover, if there are other skillsets that are associated with spindle characteristics or frequency characteristics of NREM sleep (such as delta and theta).

Slow Wave Activity (SWA): Components and Attributes

Another prominent feature of NREM sleep depicting age-related change is SWA. SWA is described as the fundamental sleep important for physical and learning performance (de Andrés, Garzón, & Reinoso-Suárez, 2011). SWA provides a measure of sleep homeostatic activity, in which increased waking (the longer one is awake) is followed by deeper and elongated sleep (Huber & Born, 2014). Slow waves are composed of periods of hyperpolarization and depolarization (Astill, 2014; Kurth et al., 2010), often occurring in parallel with sleep spindles, and defined as high-amplitude, low frequency (< 5 Hz) waves (Blake & Gerard, 1937). Slow oscillations arise from the low-frequency oscillation (1 Hz) in the membrane potential of cortical neurons and with synchronization across many neurons, the result is illustrated as slow waves seen in the surface EEG (Esser, Hill, & Tononi, 2007; Vyazovskiy et al., 2009). Slow waves are described by spectral power of the EEG between 0.25-4.5 Hz showing amplitude of the slow wave (Achermann, 2009). Over the course of a night, slow waves feature a local topographical distribution where some regions alternate between active and inactive states (Nur et al., 2011).

Similar to sleep spindles, SWA also provides both trait- and state-like attributes. Research from adulthood depicts the slow wave topography as being stable within participants and serves as a biological fingerprint (De Gennaro et al., 2005; Finelli et al., 2001). SWA also depicts inter-individual and intra-individual differences that emerge after state alterations (Ringli & Huber, 2014). For example, sleep deprivation results in increased SWA (Ringli & Huber, 2014). Extensive learning before sleep also results in global or local increases in SWA (Chen et al., 2000; Finelli, Baumann, Borbély, & Achermann, 2000; Huber et al., 2004).

SWA: Characteristics in Early Childhood

Compared to adults, children have larger SWA (Huber & Born, 2014) that dramatically changes during development, peaking before puberty and gradually declining thereafter (Kurth et al., 2010). In the first two decades, SWA shifts from occipital to frontal regions (Kurth et al., 2010; Buchmann et al., 2011b; Ringli & Huber, 2011) and this shift reflects the path of cortical maturation (Huttenlocher & Dabholkar, 1997). Kurth et al. (2010) used a cross-sectional study (2.4–19.4 years) and showed the topography of SWA changes from young children (2.4-5-year-old) to young adulthood with a regional shift of SWA along the postero–anterior axis (Kurth et al., 2010). The underlying shift from more posterior to frontal regions may underscore the development of more sophisticated cognitive processes that children develop with age (Kurth et al, 2012). In adults, SWA is most prominent in frontal brain regions (Gaudreau, Carrier, & Montplaisir, 2001), the areas of the brain that are important for sophisticated cognitive functions as working-memory and attention. These higher level cognitive functions are impaired by sleep deprivation (Gaudreau et al., 2001; Kurth et al., 2016).

Though Kurth et al. (2010) did not examine infants and toddlers, findings show local distributions of power across lower frequencies in all ages with a clear maximum over frontal

regions in adolescents. Recently, Novelli et al. (2016) used a cross-sectional study, examining all night sleep recordings in newborns to 4-years old to assess the local shift in each frequency band. Novelli et al. (2016) did not examine the observed shift of SWA that was examined in Kurth et al. (2010), however, findings showed that delta activity was primarily stable in posterior occipital regions, and other low frequencies as theta also showed a more prominent shift along the antero–posterior axis and this shift was positively correlated with age. In other words, individual low frequency bands (theta and delta) showed an occipital to frontal shift, the similar shift observed in Kurth et al. (2010).

SWA: Associations with Behavioral Performance

In adults, SWA is highly associated with memory, particularly declarative (Marshall, Helgadóttir, Mölle, & Born, 2006; Rasch & Born, 2013; Stickgold, 2005) and emotional memory (Hu, Stylos-Allan, & Walker, 2006; Payne, Stickgold, Swanberg, & Kensinger, 2008). Some research with infants between 6-to-15-months examined the effects of sleep (typically a day time nap) with various aspects of memory and language (Gómez, Bootzin, & Nadel, 2006; Hupbach, Gomez, Bootzin, & Nadel, 2009), but have yet to look at associations with SWA. Currently, only a few studies have examined behavioral associations of SWA in school age children (Astill et al., 2014; Wilhem et al 2014) and young children (Cremone, Kurdziel, Fraticelli-Torres, McDermott, & Spencer, 2017).

In addition to sleep spindles, Astill et al (2014) researched associations of SWA and motor skill (finger tapping sequence) in 10-year old's. Children had better performance (increased speed) with faster slow waves. In a similar study, Wilhem et al (2014) examined 15 children (mean age 10 years), 14 adolescents, and 17 adults and the extent to which local experience-dependent changes in SWA varied as a function of age under a visuomotor learning

task with two conditions (pre and post an all-night sleep recording). Compared to the baseline condition, SWA was elevated in children in the right parietal cortex. Astill et al. (2014) had similar findings of memory and SWA, showing accuracy increased only with the sleep condition and was most pronounced in children with more slower slow waves. In other words, the children with a lower initial performance.

The maturation of various skills as simple motor skills, complex motor skills, visuomotor skills, language skills, and cognitive control skills as a function of the topography of SWA has been studied (Kurth et al., 2012). Children with higher SWA had better performance in motor movements such as finger tapping. Recently, Cremone et al. (2017) examined the benefits of a day time nap (compared to the same amount of time spent awake (nap deprivation)) in reducing biases in attention allocation in 4 and 5-year old. All children completed a visual attention task, post nap/wake and the results indicated that increased SWA during the nap was associated with faster response time. The effects of nap deprivation on emotional attention biases were more robust for habitual nappers (children who consistently nap on a daily basis) (Cremone et al. 2017). Thus, children who are habitual nappers, when deprived of their nap, showed increased bias for emotional attention. The research findings in young children underscore the role of plasticity and maturation as being particularly pronounced during this age range with increased SWA as a prominent feature that is highly associated with behavior (Cremone et al., 2017; Kurth et al., 2012).

Given the strong associations of sleep and memory in adults (Strickgold et al., 2005) it is not surprising that the research examining infants and toddlers focuses on the benefit of habitual napping and memory (Gomez et al., 2006; Hepbach et al., 2009) and only a few studies have examined the relationship of SWA and behavior in young children. Associations of sleep

spindle characteristics with IQ and behavior are widely studied in adults and to a lesser extent school age and preschool children. In infants and toddlers these comparisons are relatively ignored, and there remains a dearth of research examining spindle and SWA characteristics and behavior in these age groups.

In addition to being understudied, infant and toddler development is typically studied from an early childhood perspective, where behavioral performance is often examined as a function of five traditional developmental domains (cognitive, fine motor, gross motor, language, and social emotional) and these domains are often examined using paper assessments and assessed during play (Lifter & Bloom, 1989). Moreover, many of these domains are not only important but are the fundamental components to learning and the development of more sophisticated cognitive functions (Kurth et al. 2012). Thus, it is surprising that research has yet to examine the associative role of both sleep spindles and SWA with these important developmental domains.

NREM Sleep in ASD

The bulk of literature in ASD and sleep examine: (a) parental stress and their child's sleep (Malow et al., 2006a); (b) sleep problems (Limoges et al., 2005); (c) REM sleep in ASD (Buckley et al., 2011; Daoust et al., 2004; Léveillé et al., 2010; Ornitz et al., 1969; Tanguay, Ornitz, Forsythe, & Ritvo, 1976); (d) sleep in ASD with and without regression (Giannotti et al., 2008; Giannotti, Cortesi, Cerquiglini, Vagnoni, & Valente, 2011); (e) adults with Asperger's' and high functioning autism (Godbout et al 2000; Limoges et al., 2005); and (f) abnormalities in sleep EEG such as epilepsy and seizures (Yasuhara, 2010). Only a few studies have examined NREM sleep in populations with various forms of autism. Given the gap of research in this area,

the reviewed research will incorporate research from adulthood to preschool with samples classified as having either Asperger's', high functioning autism (HFA), autism, or ASD.

Adults

Godbout et al. (2000) examined all night sleep in 8 individuals (range 7-53 years, mean = 22.6) with Asperger and 8 gender and age matched participants. The group with Asperger syndrome showed decreased sleep spindle density during the beginning and end of the all-night sleep recording. Similarly, Limoges et al (2005), also examined sleep spindles in adults with Asperger (n=6), HFA (n=10), ASD (n=16), and control (n=16), in an all-night sleep study. Group differences were found between the group with Asperger's' and HFA, with significantly reduced sleep spindles (during Stage 2 sleep) in the group with Asperger's', which is comparable with findings from Godbout et al. (2000). Participants with ASD had significantly less sleep spindles compared to the control participants. Other noteworthy differences are the ASD group showed increased duration of stage 1 sleep (light sleep), decreased NREM sleep (deep sleep), and fewer periods of REM sleep. The group with ASD also took longer to fall asleep, spent less time asleep, and woke up more frequently throughout the night.

Despite these findings with sleep spindles, thus far only one study has examined SWA in ASD. Recently, Rochette, Soulieres, Berthiaume, and Godbout (2018) examined delta activity, a marker of SWA in adults with ASD. Compared to a control group, adults with ASD had significantly less delta EEG activity in parieto-occipital regions and decreased delta activity in frontal to posterior regions. This suggests that individuals with ASD have decreased SWA and likely decreased S3, the stage in which SWA occurs and thus, experience less "deep sleep". Decreased SWA is associated with decreased performance on motor memory and learning

(Doucette et al., 2015), and memory consolidation, and may be associated with symptom severity in populations with ASD.

These findings show that many features of sleep are atypical for the group with ASD as well as the groups with HFA and Asperger's', which is consistent with other research suggesting the neural networks are altered in populations with ASD (Ghuman et al., 2016). Fewer sleep spindles and aberrant delta activity Rochette, Soulieres, Berthiaume, and Godbout (2018), suggest anomalies in the thalamocortical network, the source and generation of sleep spindles (De Gennerao & Ferrara, 2003). Thus, disruptions in the functional integrity, the source of NREM sleep could be why there are fewer sleep spindles in populations with ASD. One limitation across many studies with ASD are small samples (Wang et al., 2013) and moreover samples with a large age range. Research has shown that across the lifespan, immense change in SWA (Kurth et al. 2010) and sleep spindle characteristics exist (De Gennaro & Ferrara, 2003). With a large age range, it is difficult to interpret findings from a sample that include school age, puberty, adolescence, and adults (Godbout et al., 2000).

Children

Two studies have examined features of NREM sleep in children on the spectrum. Bruni et al. (2007) examined features of NREM sleep in children (7-15 years old) with Asperger syndrome (n=8), autism (n=10), and typically developing controls (n=12). Although the authors did not specifically look at sleep spindles, minor differences were found in the sleep architecture between the 3 groups (Bruni et al., 2007). The groups with Asperger's' and autism showed decreased N2 sleep, but no decreases in N3 (SWS). Sleep spindles occurred primarily during N2 and N3. Given the decreased N2 sleep, this suggests that sleep spindles are likely decreased in the groups with Asperger or autism. Tessier et al. (2015) is the only study to examine sleep

spindle characteristics (density) in 13 school-aged children (mean=10.23 years) with HFA. The HFA group had fewer fast spindles than the control group. These findings are on par with the adult literature also showing reduced spindles in populations with ASD (Godbout et al., 2000; Limoges et al., 2005). Although research in these populations shows decreased sleep spindles, SWA has yet to be fully examined in both adults and children with ASD. Though limited, these results suggest that the observed differences of cortical topography between control and ASD/HFA group may be a fruitful avenue to further detect differences in younger populations.

NREM Associations with Behavioral Performance in ASD

Of the few studies to examine features of NREM sleep (sleep spindles and/or SWA) two have examined associations with primarily memory and other skillsets. Limoges et al., 2013 examined 31 adults (17 ASD, 14 TD) during an all-night sleep recording and assessed participant's daytime performance on a measure of IQ to investigate if sleep quality impacts cognitive functioning. Participants with ASD showed signs of poorer sleep that were highly correlated with various aspects of motor output on non-verbal performance tasks. For example, there was a significant negative correlation between slow-wave sleep (SWS) and learning a sensory-motor procedural memory task. Sleep spindles were negatively associated with both the number of trials needed to learn a sensory-motor procedural memory task, and reaction time in that task. These findings suggest that fewer sleep spindles are associated with more trials to learn a task, increased reaction times to complete the task, and increased errors when performing the task.

In Tessier et al. (2015), another focus of the study was to examine features of NREM sleep and associations with IQ. The authors examined two variables within NREM, frontal and central sleep spindles, and correlations between intelligence measures (Wechsler Intelligence

Scale for Children-IV (WISC-IV) test). The control group showed a negative correlation between verbal IQ and frontal (slow) spindle density. The ASD group showed a negative correlation of verbal and full-scale IQ with central (fast) spindle density, whereas the control group showed a positive correlation with spindles in the central region (fast) with verbal IQ.

Although the research examining NREM sleep is limited, evidence exists that NREM activity is altered in the sleep state in individuals on the autism spectrum. Research with adults shows decreased sleep spindles while research with young children shows both positive and negative relationships in both slow and fast spindles. No study has yet to examine features of NREM sleep in infants/toddlers with or at-risk of ASD. As such, it is unclear if infants/toddlers that have or at-risk of ASD have atypical spindle characteristics or SWA. It is also unknown if features of sleep spindles or SWA are associated with IQ or behavioral performance to track development in this age span. Any potential identification of sleep spindle and SWA patterns in infants/toddlers with or at-risk of ASD would represent progress toward a biomarker for the disorder. Moreover, no studies have examined features of NREM sleep as a possible risk marker in infants/toddlers at-risk of ASD. With a risk marker in place, this could make early diagnosis more reliable (Varcin & Nelson, 2016), and thus suggest access to early intervention before symptoms are present at the behavioral level.

Infant/Toddler NREM Sleep: Candidate Risk Marker of ASD

The inception of a biomarker used to differentiate infants at-risk of ASD, before the onset of symptoms, may be a fruitful strategy for research directed at understanding the neurological underpinnings of ASD (Jeste, Frohlich, & Loo, 2015; Varcin & Nelson, 2016). Compared to traditional screening measures, a biomarker suggests risk well before behavioral symptoms are apparent, whereas traditional screeners and assessments rely on the presence or lack of specific

behaviors and symptoms. Children develop at various rates, and due to this variation, traditional screeners sometimes fail to capture the nuances of development (Varcin & Nelson, 2016). As such, a biomarker(s) signifying risk before the onset of symptoms, suggesting that EI is needed, and in turn, reducing symptom severity and increasing cognitive outcomes would be highly beneficial (Dawson et al., 2012).

A biomarker as described by the National Institutes of Health (NIH), is "a characteristic that is objectively measured and evaluated as an indication of normal biological processes, pathogenic processes, or pharmacological responses to an intervention," (NIH, 1998). The term biomarker has a biological basis that can be delineated into subsets for example, stratification and target engagement (McPartland, 2016; Walsh, Elsabbagh, Bolton, & Singh, 2011). Stratified markers classify populations into subgroups based upon relevant treatments while a target engagement marker provides evidence that the treatment or intervention is altering brain activity (McPartland, 2016; Zhao, Modur, Carayannopoulos, & Laterza, 2015). A biomarker can also be a screening marker used to predict risk status (Varcin & Nelson, 2016). A risk biomarker of ASD in sleep could be differences in the sleep EEG during NREM sleep such as differences in sleep spindle characteristics or reduced or delayed SWA. The proposed study seeks to assess the extent to which differences are found in the 12-30-month age range that may be used to detect risk of ASD well before symptoms become apparent at the behavioral level.

Due to various reasons such as clinical heterogeneity (Jeste, Frohlich, & Loo, 2015), poor participant descriptions (Wang et al., 2013), and methodological inconsistencies such as sample size and task dependent states (Wang et al., 2013), research has yet to establish a biomarker of ASD. There is sufficient need for research improving the conditions to establish a biomarker capturing a larger subtype within the population (Walsh et al., 2011) and moreover, showing

clinical promise in denoting risk, malleable to interventions, and developmental trajectories. NREM sleep is put forth as a potential avenue to further investigate a risk marker of ASD. Sleep with EEG in populations with ASD are understudied and to date, no studies have examined hdEEG during sleep, known to reflect maturational and regional differences for detecting risk for ASD.

This chapter examined cortical activity and development during sleep with a focus on NREM sleep. Despite the prevalence of sleep literature in adults, the transition from infancy to toddlerhood has yet to be fully examined. Research shows that NREM sleep follows a maturational pathway that is highly associated with behavior. Features of NREM sleep are shown to be implicated in populations with neurodevelopmental disorders, including ASD. There is a growing body of research directed at understanding the underpinnings of ASD in hopes of identifying a risk biomarker(s) of ASD. Although many potential biomarkers are put forth, currently no definitive biomarker for ASD exists. With a large body of research motivated to identify biomarker(s) of ASD, it is likely that a marker will be multifaceted to capture the heterogeneity of ASD (Jeste, Frohlich, & Loo, 2015) and the study of NREM sleep has potential as an assay to detect differences in the infant topography as a potential biomarker of ASD.

Present Study

The purpose of this study is to characterize brain network dynamics (activity patterns) during sleep in infants/toddlers diagnosed with ASD, at-risk of ASD, and typically developing infants/toddlers. Assessing the effectiveness of sleep as an assay to detect differences in the infant EEG is a first step in characterizing the topography of NREM sleep. Potential differences detected in NREM sleep may provide insight to the screening process and the early identification of ASD as a biomarker to distinguish risk of ASD.

Aims

Here, the aim is twofold, the first is to characterize the topography of sleep during a day-time nap in infants/toddlers 12-30 months of age with ASD, at-risk of ASD, and typically developing infant/toddlers. By elucidating the role of sleep spindles and dominant rhythms during NREM in this age group, provides a useful baseline marker of neurodevelopment to distinguish typical development from atypical development. Research shows that sleep spindles correlate with maturational changes in development and are associated with IQ (Fogel & Smith, 2011; Tessier et al. 2015), however, it is unknown if these features and other features of NREM sleep are associated with other traditional outcomes of early development that may be more insightful for the study of early development. Topographical and spectral characteristics of sleep spindles, SWA, and other oscillatory features may have an underlying role in development. Thus, the second aim is to differentiate oscillatory features of NREM sleep with two widely used norm-referenced measures in early child development (The Mullen Early Scales of Learning (MSEL) and the Vineland-II). The specific research questions to be addressed are:

Research question 1a. What are the physiological activity patterns of NREM sleep (sleep spindles and SWA) in infants/toddlers 12-30 months of age?

Research question 1b. What physiological activity patterns of NREM sleep are associated with development in infants/toddlers 12-30 months of age?

Research question 2a. Given the physiological activity patterns of NREM sleep in infants/toddlers, what patterns of NREM sleep are associated with ASD?

Research question 2b. Given the physiological activity patterns of NREM sleep and associations with development in infants/toddlers, what features of NREM sleep are associated with development in infants/toddlers with ASD?

CHAPTER THREE: METHODOLOGY

Data from the Early Development and Sleep study (EDS) were used to explore the sleep architecture (patterns) across infancy and toddlerhood in a sample of typically developing children and children with or at-risk for an autism spectrum disorder (ASD). The EDS study used quantitative methodology with a cross sectional design to examine TD infants, infants/toddlers with ASD, infants/toddlers at-risk for ASD to explore topography and features of the infant/toddler sleep architecture during a midday nap. High density electroencephalogram (hdEEG) data from the midday nap was used to examine features of non-rem (NREM) sleep and correlated these features with the assessments completed during the home visit. This chapter provides an in-depth discussion of the study's research methods, including participant sample, and data collection, processing, and analysis.

Participants

Recruitment. Families of infants and toddlers were recruited using the following outlets: (1) Flyers in local hospitals, pediatric offices, childcare centers, libraries, and Starbucks. (2) Mass email sent through the UNC mass email system to notify students, staff, and faculty that may be interested in participating. (3) Autism registry at the Carolina Institute for Developmental Disabilities (CIDD). (5) Children's Developmental Service Agency (CDSA) in Wake and Durham/Orange Counties. (6) Newspaper (INDY), (7) Radio (NPR), (8) Facebook, (9) the Frohlich Lab website, (10) the Carolina Center for Brain Stimulation website, and (11) the Chapel Hill Transit Authority.

Inclusion and Exclusion Criteria. Families with infant(s)/toddler(s) (males and females) between 12-30 months of age, that were typically developing (TD), identified as having ASD, at high familial risk for ASD (infant sibling), or at-risk based upon delays in language (i.e., no words at 18 months, limited vocalizations at 12 months) or social communication were included in this study. Infants and toddlers with the following characteristics were excluded from the study: diagnosis of epilepsy, identified disability other than ASD, or severe visual or motor impairments that impede participation.

Screening. Once parent(s) indicated their interest in participating by email or phone, they participated in a phone screening about their child's development. The phone screening was only used to determine if their child was eligible for the study. The initial screening call was approximately 10-20 minutes. Interested families were screened via telephone with the First Year Inventory (FYI) version 3.1B or the Modified Checklist for Autism in Toddlers-Revised (MCHAT-R). The FYI was administered to families that had an infant 12-15 months of age and the MCHAT-R was administered to families with a toddler 16-30 month of age. Based upon the parent(s) responses to the FYI/MCHAT, they were then informed if their child was eligible for the study.

Study Sample

Participants were 30 (14 males, 16 females) typically developing (TD) infants/toddlers (mean age= 20 months, SD=5.19, range=12-30), and 4 (3 male, 1 female) toddlers with ASD (mean age= 25.25 months, SD=3.40, range=22-30), and 3 (2 male, 1 female) infants/toddlers at-risk (mean age= 19 months, SD=9.54, range=13-30). All of the at-risk participants scored as having moderate-to-severe concern for ASD (determined by the ADOS-2 described below) and were grouped with participants with a formal diagnosis (also scoring moderate-to-severe) and

from here on, referred as ASD. All participants (TD and ASD) were of healthy weight and height for age (i.e., 5th–85th percentile BMI) and in all instances but one (participant with ASD), born full term. Two participants (TD) did not sleep during the lab visit and one participant had an excessive rocking artifact (from the car seat) that created noise in the EEG signal (TD) and thus, these 3 participants were excluded from analysis. Participant and maternal demographics for the remaining 34 participants (27 TD, 7 ASD) are shown in Table 1.

Table 1: Child and Maternal Demographics

Participants (n=34)		
	TD (n=27) n (%)	ASD (n=7) n (%)
Child Gender		
Female	15 (55.6)	2 (28.5)
Male	12 (44.4)	5 (71.5)
Race/Ethnicity		
White	21 (77.8)	4 (57.1)
Black/African	3 (11.1)	3 (42.9)
American	1 (3.7)	0 (0.0)
Asian/Pacific Islander	1 (3.7)	0 (0.0)
Other/Multiracial	1 (3.7)	0 (0.0)
Hispanic/Latino		
Marital Status		
Married	24 (88.9)	4 (57.1)
Never Married	2 (7.4)	1 (14.3)
Separated	0 (0.0)	1 (14.3)
Divorced	0 (0.0)	1 (14.3)
Widowed	1 (3.7)	0 (0.0)
Household Income		
Less than $25,000	4 (14.8)	2 (28.5)
$25,00 to $49,999	7 (26.0)	1 (14.3)
$50,000 to $74,999	6 (22.2)	3 (42.9)
$75,000 to $99,999	4 (14.8)	1 (14.3)
Greater than $100,000	6 (22.2)	0 (0.0)
Maternal Education		
Associates/Vocational	2 (7.4)	1 (14.3)
4-year degree	11 (40.7)	2 (28.6)
Graduate/professional	14 (51.9)	4 (57.1)

Procedures

Once the child was determined to be eligible for the study, the first visit (home visit) was scheduled at a time that was convenient for both the child and their parent(s). Both the home visit and lab visit are described in further detail, below. Participants received $75 as compensation for participating in the study. Participants underwent a home visit ($35) and a lab visit ($40, explained in more detail below). If for any reason the participant only completed one visit (for example, the home visit but not the nap visit), they received a prorated payment of $35.00.

Home Visit. All assessments were conducted during the initial home visit. All home visits and assessments were completed by myself, Jessica Page as I have received extensive clinical and research training on the following measures used in the current study. The purpose of the home visit was to assess the child's development at the behavioral level. Each home visit was approximately 2 - 2 ½ hours. Once the parent(s) gave consent, all parents received a sleep diary (SD) to document their child's sleeping behaviors, beginning a week prior to the lab visit. Families completed and brought the SD to the lab visit. All participants underwent the following assessments.

Measures

The Autism Diagnostic Observation Schedule interview (ADOS-2, Lord et al., 2012*)* is a semi-structured, standardized assessment of communication, social interaction, play, and restricted and repetitive behaviors. The ADOS-2 is considered the "gold standard" used to assess possibility and severity of ASD by eliciting behaviors directly related to a diagnosis of ASD. The ADOS-2 has five modules: Toddler, Modules 1, 2, 3, and 4. Given the age and language level of the infants in the study, all participants completed the toddler module.

The Mullen Early Scales of Learning (MSEL, Mullen et al., 1998) is a standardized norm-reference tool designed to measure cognitive development in individuals birth to 68 months. The MSEL's five domains include: Gross Motor (GM), Fine Motor (FM), Visual Reception (VR), Expressive Language (EL), and Receptive Language (RL). Gross Motor scores were not reported as all children performed within a standard deviation on Gross Motor. T-scores were provided for the domain scales, and the Early Learning Composite (overall standard score) was used to determine cognitive functioning.

The Vineland Adaptive Behavior Scales – 2nd Edition (VABS-2, (Sparrow, Cicchetti, & Balla, 2005) is a standardized norm-referenced tool designed to measure daily functioning in individuals from birth to ninety years of age. The five VABS-2 domains include: Communication, Daily Living, Socialization, Motor Skills, and Maladaptive Behaviors. The parent(s) of the participant completed the interview version of the VABS. The interview was chosen over the survey to allow for further prompting about the child's development. In the event the parent was unsure about how to answer an item, concrete examples were provided to help clarify the question. T scores are provided for the domain scales, and the overall composite was used to determine adaptive functioning.

The *Background Information Questionnaire (BIQ)* was designed by the research group to tap demographic information about the child participants in the study and their families. The present study used the following variables from the BIQ: Child age and gender, Parents Marital Status, Household Income, and Maternal Education.

Lab visit. The parent(s) and the participant were encouraged to complete the lab visit within two weeks of the initial home visit. The parent(s) and participant arrived 30 minutes before the participant's typical nap time, which allowed the participant to become acclimated to

the new setting. Prior to the participant's nap, the parent(s) returned the SD and if for some reason the parent(s) forgot or lost the SD, they had the opportunity to complete the SD during the participant's nap visit. Participants underwent a nap recording with surface high-density electroencephalography (hdEEG, 124 or 128 electrodes) recorded using NetAmps 410 (Electrical Geodesics Inc. EGI, Eugene, OR). The circumference of the participant's head dictated whether the participant wore the 124 (44-47 cm) or a 128 (47-51 cm) electrode net. The Geodesic Sensor Net is a widely used EEG net that was securely placed on the infant's head (like a swim cap) prior to falling asleep. Participants napped in either a modified car seat, pack n' play, in a bed, or were held by his or her parent. The participants' nap (sleeping arrangement) was determined by the parent and the child's typical sleep routines. On average, participants napped duration was 84.41 ± 4.2 SEM minutes with no group differences in the amount of time napped. Participants were under constant supervision and all wires were kept away from the face and neck.

Collected EEG and Components. EEG data was continuously collected while the child transitioned form wake to sleep. Once the EEG data was collected, data underwent frequency analyses by translating the time resolution into a frequency resolution, which allows for further classification (Banaschewski & Brandeis, 2007; Fröhlich, 2016). The EEG was classified into waveforms according to their dominant frequency (Hz), amplitude (mV), shape, and scalp distribution (Banaschewski & Brandeis, 2007). Waveforms are a prominent feature in the EEG, and the most common are sinusoidal (sound waves) measured from peak to peak with frequency (Hz), power (spatial), and phase describing the properties of signals (Simon & Wallace, 2016). Frequencies were delineated into the following waveforms: gamma γ (> 30 Hz), beta β (17-30

Hz), sigma σ (10-16 Hz), theta θ (4-8 Hz), and delta δ (0.5-4 Hz). The next section describes the preprocessing of the raw EEG data which was used for analysis and sleep scoring.

Preprocessing. EEG was digitized at 1000 Hz (bandpass 0.1 to 200Hz) and referenced to the vertex (Cz). The EEG was resampled offline to 250 Hz and preprocessed using the PREP pipeline (Bigdely-Shamlo, Mullen, Kothe, Su, & Robbins, 2015). The PREP pipeline is a data reduction technique to remove line-noise, including robust average referencing (bad channel removal), and then band-pass filtered between 0.5-40 Hz. The bandpass filtered out line noise that was below 0.5 Hz (remover drift) or above 50 Hz. Brain activity above 30 Hz is considered to be in the gamma range which is typically not present during sleep. Artifacts were rejected based upon visual scoring and semiautomatic artifact removal (Kurth, 2010).

Analysis of the EEG. A Fast Fourier Transformation (FFT) (Cooley & Tukey, 1965) and spectral analysis (Achermann, 2009) was used for a more objective measure of the sleep staging and the development of spectrograms (a visual color map used to denote the spectrum of frequencies of a signal, Achermann, 2009**).** Using FFT, the signal was decomposed into frequency components for the analysis of EEG power in distinct frequency bands over space and time (Cooley & Tukey, 1965). The frequency resolution was based on the analyzed window of short durations, which creates 4- second epoch windows giving a frequency resolution of 0.25 Hz. This then created a sleep scoring epoch of 20 seconds which was averaged across 5 consecutive 4-s windows, and then used for sleep scoring. Based upon the EEG and the presence of oscillations, specific frequencies, and the guidelines set forth by the American Academy of Sleep Medicine standard criteria (AASM), sleep was scored and staged using 4- s sequential windows that were combined together to create 20 s epochs (Iber et al., 2007).

Sleep Scoring. The sleep EEG was scored visually (qualitatively) by two expert scoreres for sleep stages (20 s epochs, F4A1, C4A1, O2A1) in accordance to the AASM (Iber et al., 2007). Scorers were myself and a postdoctoral student in the Frohlich lab with extensive training on the AASM sleep scoring rules and considered reliable. All recordings were scored separately and then compared. In 95% of the cases there was full consensus and any discrepancies were resolved by mutual agreement. Scoring and analysis focused on Non-rapid eye movement (NREM), defined according to standard criteria (Rechtschaffen and Kales, 1968; Feinberg and Floyd, 1979). The EEG data lacks electromyogram (EMG, muscle movement) and some participants lacked electrooculography (EOG, eye movement), thus, the current study focused exclusively on NREM sleep. Next, quantitative assessment of the sleep EEG (Boostani, Karimzadeh, & Nami, 2017) including spectral analysis was performed for all channels using Fast Fourier Transform (FFT) (Hanning window, 20s epochs, average of five 4-s windows yielding a frequency resolution of 0.25 Hz). Based on the spectral profile, subsequent analyses were restricted to specific frequency bands, including slow waves (1–4.5 Hz), theta (4.75–7.75 Hz), alpha (8–9.75 Hz), sigma (10–16 Hz), and beta (20–25 Hz).

Measuring Sleep Spindles. Different methods are used to detect and quantify sleep spindles. One way is by examining the sigma frequency band (10-16 Hz) in the spectral analysis of NREM sleep. Though objective, this method does not allow for distinction between background EEG activity (noise) and spindle activity (De Gennaro & Ferrara, 2003). Furthermore, this measure does not distinguish between spindle features as duration and amplitude (spindle power) and density (number/min). To obtain these different spindle measures, an automatic spindle detection algorithm was used (Ferrarelli et al., 2007). To evaluate each sleep spindle detected by the algorithm (Ferrarelli et al. ,2007), the raw signal for each channel

was band-pass filtered between 10-16 Hz in consecutive 20-second epochs. To identify a spindle, the threshold relative to the mean signal amplitude, with an amplitude fluctuation in the time series exceeding an upper threshold was used. Sleep spindles from the filtered signal for each EEG channel, was based on the upper threshold (6 times the mean of filtered signal) and lower threshold (2 times the mean of filtered signal) amplitude criteria. These lower and upper thresholds were used for the entire time series for each channel (124 electrodes), as these thresholds showed the best match between visual and automatic detection and have been used in previous studies examining age related changes in spindle characteristics (McClain et al., 2016). For each detected spindle, the frequency, amplitude, duration, maximal amplitude (local maximum above the threshold), and density were analyzed.

Statistical Analysis

To address *all* research questions (1a, 1b, 2a, and 2b) statistical analyses were conducted using R version 3.28.18 (R, Inc., Boston, MA) and Matlab 2016a (Mathworks, Natick, MA). The first research question asked **1a. What are the physiological activity patterns of NREM sleep (sleep spindles and SWA) in infants/toddlers 12-30 months of age?** Quantitative analysis of sleep EEG was achieved by transforming the EEG signal into the following frequency domains, delta (.5- 2.0), slow waves (1–4.5 Hz), theta (4.75–7.75 Hz), alpha (8–9.75 Hz), sigma (10–16 Hz), and beta (20–25 Hz). Sleep spindles were described as number of spindles per unit time, duration, location, and the amplitude of their constituent sine waves. A median (Mdn) split of age was used to create two groups, younger (< 20 months) and older than 20 months (> 20 months). Table 2 below shows the distribution of age which was formed for the analysis of age-related spectral features and computation of topographic maps to address research question 1 (a and b).

Table 2: Median-age Split

	Participants (n=27)	
	< 20 Months n (%)	>20 Months n (%)
Gender		
Female	8 (29.6)	7 (26.0)
Male	6 (22.2)	6 (22.2)
Race/Ethnicity		
White	10 (37.8)	11 (40.7)
Black/African American	2 (7.4)	1 (3.7)
Asian/Pacific Islander	0 (0.0)	1 (3.7)
Other/Multiracial	1 (3.7)	0 (0.0)
Hispanic/Latino	0 (0.0)	1 (3.7)

A two-sample t-test was used to compare differences between the two groups (<20 months, > 20 months). To control for multiple comparisons across electrodes, non-parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Nichols & Holmes, 2001) as previously applied in high-density EEG studies (Huber et al., 2016; Lustenberger et al., 2015; Plante et al., 2016). This approach is used for analyses with low degrees of freedom, for example single subject experiments or multi-participant designs (Nichols & Homes, 2001). The non-parametric approach employed a local pooled variance estimate to identify clusters. Clusters that were at least equal or above the 95th percentile on either side (minimal and maximal clusters) were considered significant and marked as significant in the topographical plots (p-value < 0.05).

1b. What physiological activity patterns of NREM sleep are associated with development in infants/toddlers 12-30 months of age? EEG data analysis from the two age groups detailed in 1a, were compared with electrode-wise two sample t-tests. Correlations of the sleep EEG, MSEL domains (Fine Motor, Visual Perception, Receptive Language, Expressive Language), and VABS domains (Communication, Socialization, and Motor Skills) were performed using Pearson correlation. To control for multiple comparisons across electrodes, non-

parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Nichols & Holmes, 2002) as previously applied in high-density EEG studies (Huber et al., 2006; Lustenberger, Murbach, et al., 2015; Plante et al., 2016). Clusters that were at least equal or above the 95th percentile on either side (minimal and maximal clusters) were considered significant and marked as significant in the topographical plots (p-value < 0.05).

Descriptive statistics (mean and standard deviation (SD)) and t-tests were used to describe participant and maternal demographics and examine group differences on the VABS and MSEL. To control for multiple comparisons and decrease the likelihood of Type I error, Bonferroni correction was used. Though Bonferroni correction is conservative, it is often used with a small number of comparisons and thus, all tests were controlled with this approach (McDonald, 2014). Correlations with the developmental behavioral assessments MSEL domains (Fine Motor, Visual Perception, Receptive Language, Expressive Language) and VABS domains (Communication, Socialization, Daily Living, and Motor Skills) were performed using Pearson correlation.

To address the second research questions (2a and 2b), primary and secondary analyses were used and further outlined below. **(2a) Given the physiological activity patterns of NREM sleep in infants/toddlers, what patterns of NREM sleep are associated with ASD?** All participants identified by their caregiver as either having an ASD diagnosis or at-risk met criteria based on the Autism Diagnostic Observation Schedule 2nd Edition (ADOS-2). Infants/toddlers with ASD were grouped together and compared to a group of age matched typically developing (TD) participants. Data for the primary analysis from the two groups (outlined in Table 3) were used for the analysis of spectral features, computation of topographic maps, and descriptive statistics (mean and SD) to address research question 2 (a and b).

Table 3: Participant Age Matched Comparison

Groups			
TD		ASD	
Age (months)	Gender	Age (months)	Gender
13	Female	13	Female
14	Male	14	Male
22	Female	22	Male
24	Male	24	Male
25	Female	25	Female
30	Male	30	Male
30	Male	30	Male

For the primary analyses, quantitative analysis of sleep EEG was achieved by transforming the EEG signal into the following frequency domains, delta (.5- 2.0), slow waves (1–4.5 Hz), theta (4.75–7.75 Hz), alpha (8–9.75 Hz), sigma (10–16 Hz), and beta (20–25 Hz). The EEG data from the ASD and TD group were compared with electrode-wise paired and unpaired t-tests. Correlations of the sleep EEG from the TD and ASD groups were performed using Pearson's r product-moment correlation. To control for multiple comparisons across electrodes, non-parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Nichols & Holmes, 2002) as previously applied in high-density EEG studies (Huber et al., 2016; Lustenberger et al., 2015; Plante et al., 2016). Clusters that were at least equal or above the 95th percentile on either side (minimal and maximal clusters) were considered significant and marked as significant in the topographical plots (p-value < 0.05).

Given the small participant sample, a secondary analysis was also computed to further explore the benefits of an increased sample size. Though a limitation of this method is non-independent samples, the resampling with replacement of participants was used for further exploration of the data. Thus, some participants from the ASD group were doubled and used twice. This approach is similar to other resampling or imputation methods such as mean or

regression imputation. Given that the analysis required an age matched participant, the resampling of participants required the whole unit (in this case the data from an age matched participant) to be used twice. Table 4 outlines the secondary analysis (resampling of participants).

Table 4: Resampled Participant Age Matched Comparison

Groups			
TD		ASD	
Age (months)	Gender	Age (months)	Gender
13	Female	13	Female
13	Female	13	Female
14	Male	14	Male
22	Female	22	Male
22	Female	22	Male
24	Male	24	Male
24	Male	24	Male
25	Female	25	Female
30	Male	30	Male
30	Male	30	Male

For the secondary analysis, quantitative analysis of sleep EEG was achieved by transforming the EEG signal into the following frequency domains, delta (.5- 2.0), slow waves (1–4.5 Hz), theta (4.75–7.75 Hz), alpha (8–9.75 Hz), sigma (10–16 Hz), and beta (20–25 Hz). The EEG data from the ASD and TD group were compared with electrode-wise paired and unpaired t-tests. Correlations of the sleep EEG from the TD and ASD groups were performed using Pearson's r product-moment correlation. To control for multiple comparisons across electrodes, non-parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Nichols & Holmes, 2002) as previously applied in high-density EEG studies (Huber et al., 2016; Lustenberger et al., 2015; Plante et al., 2016). Clusters that were at least equal or above the

95th percentile on either side (minimal and maximal clusters) were considered significant and marked as significant in the topographical plots (p-value < 0.05).

(2b) Given the physiological activity patterns of NREM sleep and associations with development in infants/toddlers, what features of NREM sleep are associated with development in infants/toddlers with ASD? Data from the two groups (outlined in Table 3) were used for the topographic maps and correlations with performance. Pearson correlations were computed with the sleep EEG (findings from 2a), MSEL (FM, VP, RL, EL), and VABS (Communication, Socialization, and Motor Skills). To control for multiple comparisons across electrodes, non-parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Huber et al., 2016; Lustenberger et al., 2015; Nichols & Holmes, 2002; Plante et al., 2016). Clusters that were at least equal or above the 95th percentile on either side (minimal and maximal clusters) were considered and marked as significant in the topographical plots (p-value < 0.05). To examine participants performance descriptive statistics (mean and standard deviation (SD)) were used. Due to the small sample size and the non-normality of the distributed data, p values were obtained using Mann-Whitney U to describe participants performance on the MSEL and VABS. To control for multiple comparisons, Bonferroni correction was used.

To further explore the benefits of an increased sample size, the resampling of participants outlined in Table 4 was used for secondary analyses of topographic maps and correlations with performance. Pearson correlations were computed with the sleep EEG (findings from 2a), MSEL (FM, VP, RL, EL), and VABS (Communication, Socialization, and Motor Skills). To control for multiple comparisons across electrodes, non-parametric statistical mapping (SnPM) with suprathreshold cluster analysis was used (Huber et al., 2016; Lustenberger et al., 2015; Nichols & Holmes, 2002; Plante et al., 2016). Clusters that were at least equal or above the 95th

percentile on either side (minimal and maximal clusters) were considered and marked as significant in the topographical plots (p-value < 0.05). Descriptive statistics (means and SD) and p values were obtained with Mann -Whitney U to describe participants performance on the MSEL and VABS. To control for multiple comparisons, Bonferroni correction was used.

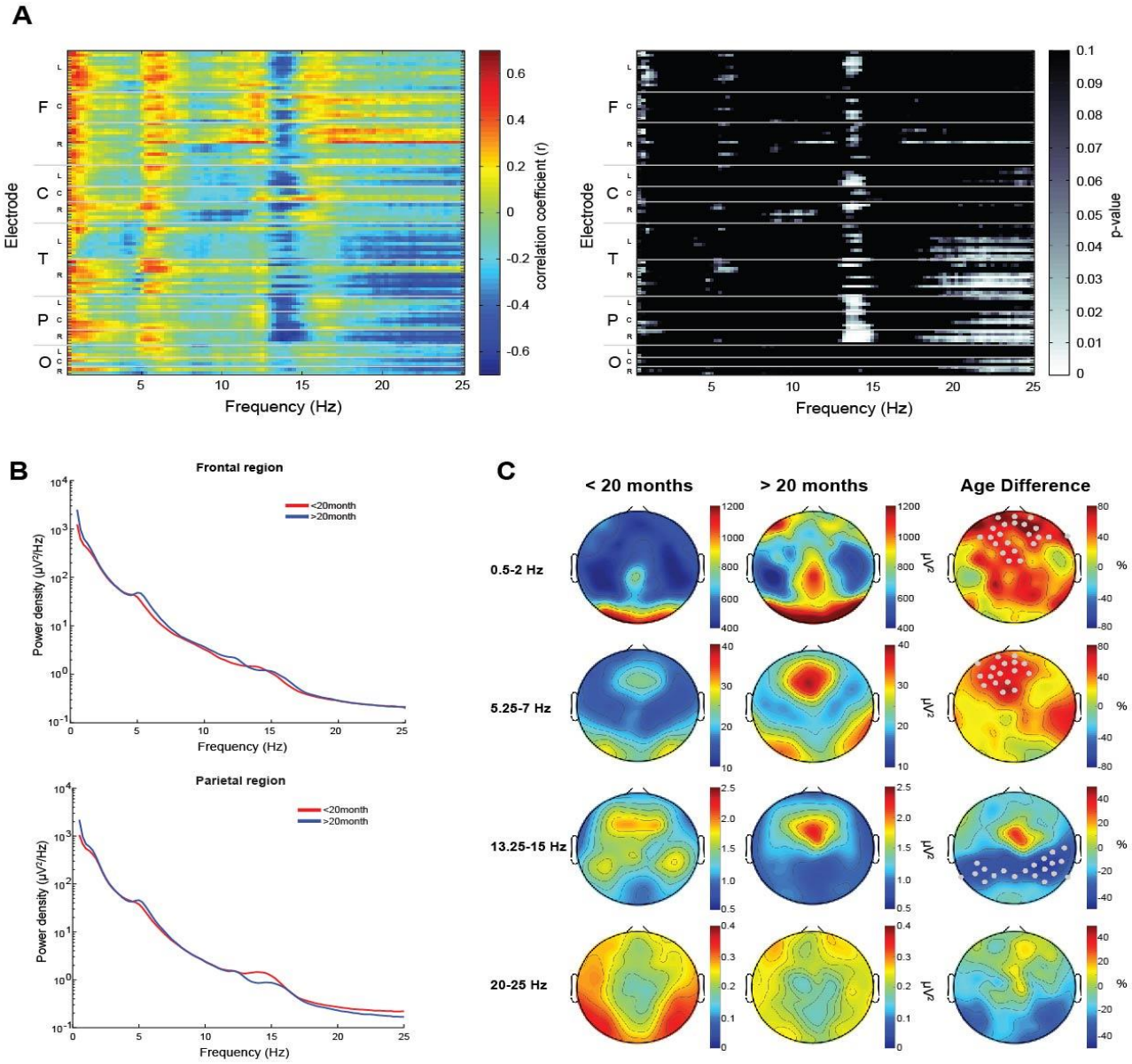
CHAPTER FOUR: RESULTS

The aim of this study was to characterize development and NREM sleep from infancy to toddlerhood. It examined features of NREM sleep and associations with behavior in infants and toddlers with typical development and those with or at risk for ASD. In this chapter, the results of each research question are presented in succession.

Research question 1a. What are the physiological activity patterns of NREM sleep (sleep spindles and SWA) in infants/toddlers 12-30 months of age?

To better examine age-related changes in the 12 - 30-month range, a median- split of age was formed (see Table 2). Despite the unequal gender distribution in both the younger and older groups, NREM spectral features did not differ by gender in either group and was not statistically significant. The physiological changes in the spectral features of NREM sleep show changes in power, location, and age with various differences in sleep spindles and SWA. Spectral changes across all investigated frequencies (0.5 - 25 Hz) are shown in Figure 1.

Figure 1: Spectral correlation during non-rapid eye movement (NREM)



(A) Correlation heat map of NREM spectral power values during NREM sleep and age. Left: Pearson correlation coefficients are depicted with warm and cold colors for positive and negative correlations, respectively. Right: p-values. (B) Spectral density plot for frontal and parietal electrode sites. Participants separated by median-split age group, younger infants (< 20 months) shown in red and older infants (> 20 months) are shown in blue. (C) Topographical illustration of specific frequency bands that showed correlations with age (shown in Panel A). Values are plotted on the planar projection of the hemispheric scalp model. Electrodes that showed a

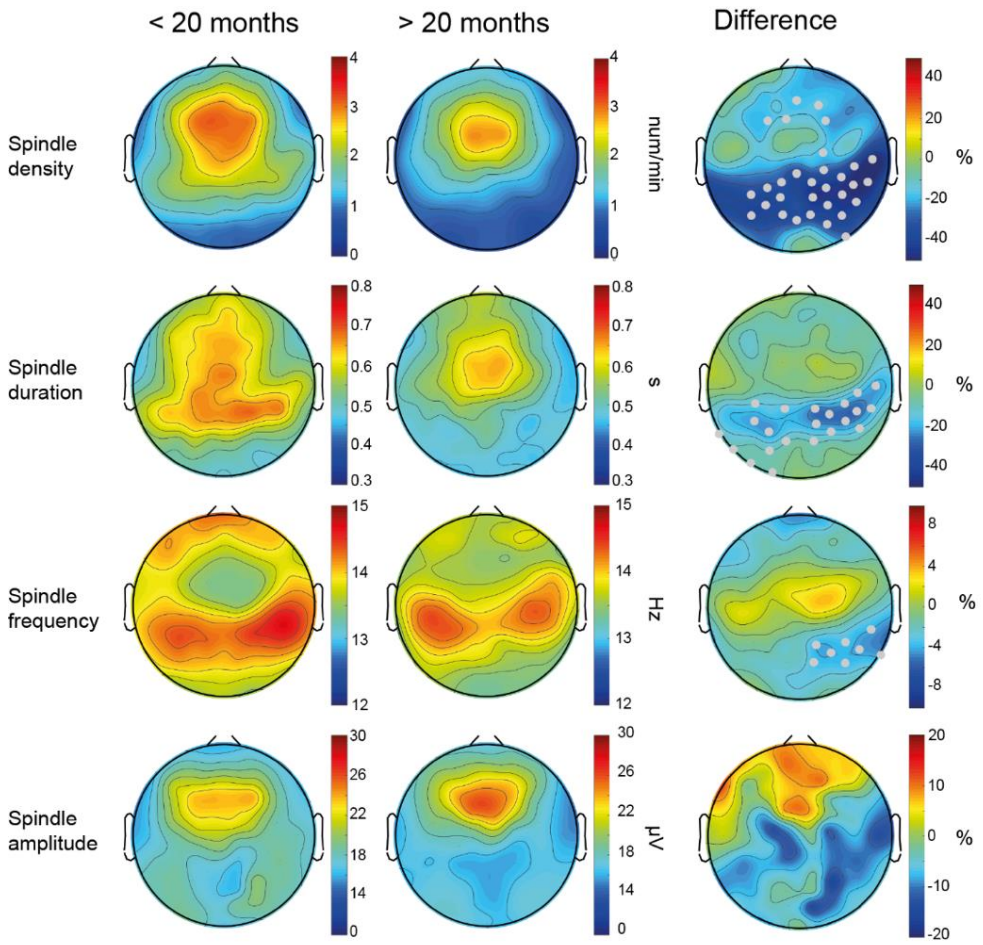
significant difference between age groups (unpaired t–test) after non-parametric statistical mapping (SnPM) are denoted with grey dots in the difference plot (Older - Younger). [1]

Sleep Spindles. A distinct finding is the change in the spindle frequency band (10-16 Hz) that was characterized by a decrease in power in posterior regions (Figure 1). The decrease in posterior regions reflects a shift from a single spindle peak in the younger children (12-20 months, approximately 14 Hz) to a double peak (approximately 12 and 16 Hz) with a trough around 14 Hz in older children (20 – 30 months). Visual inspection of the parietal spectra (Figure 1b) showed a clear spindle peak. For all participants in the older group, except for one, visual inspection of individual spectral plots (see Appendix B) showed a clear double spindle peak. Two of 15 participants in the younger group had a clear double peak. Importantly, one child in the older group did not show a distinct spindle peak in the spectral density plots for both frontal and parietal areas. Figure 1c highlights the topography of significant differences between the median split of age and the statistical comparison of spectral changes (unpaired t-test). Power spectrum changes in the spindle frequency range (Figure 1c) reflect a significant decrease in both spindle density and spindle duration (Figure 2) at posterior regions for the older group.

To better understand which spindle characteristics changed with age, an automatic spindle detection (Ferrarelli et al., 2007) was performed and a summary of results are provided in Figure 2. Similar to the spectral changes in the spindle frequency range shown in Figure 1c, spindle density (number/min) and duration significantly decreased in posterior regions. Moreover, mean spindle frequency also decreased in posterior regions. These findings suggest a reduction of spindle power with age. Thus, the reduction of spindle power with age appears to be explained by a decrease in the number and duration of sleep spindles in this age range.

[1] This is a pre-copyedited, author-produced version of an article accepted for publication in *Sleep* following peer review. The version of record (Page, Lustenberger, & Frohlich, 2018) is available online at: https://doi-org.libproxy.lib.unc.edu/10.1093/sleep/zsy024.

Figure 2: Topography of sleep spindle characteristics for younger and older groups



Topographical illustration of distinct frequency bands. Values plotted on the planar projection of the hemispheric scalp model. Electrodes that showed a significant difference between age groups (unpaired t–test) after non-parametric statistical mapping (SnPM) and suprathreshold cluster analysis are marked with grey dots in the difference plot (Older - Younger).[2]

SWA. Other significant findings in this study were differences in SWA. SWA showed an increase with age and positive correlations of power in low delta (.5-2 Hz), theta (5.25-7 Hz), sigma (11-15 Hz), and beta (20-25 Hz) frequency bands (the frequencies shown to change with age, observed in Figure 1c). One possible explanation for this finding is due to the up-states of SWA which are thought to facilitate sleep spindles and occur in succession. Thus, slower SWA

[2] This is a pre-copyedited, author-produced version of an article accepted for publication in *Sleep* following peer review. The version of record (Page, Lustenberger, & Frohlich, 2018) is available online at: https://doi-org.libproxy.lib.unc.edu/10.1093/sleep/zsy024.

(<1.25 Hz) is likely present with slower spindles, whereas faster SWA (>1.25 Hz) is typically observed with faster spindles.

NREM Findings. Other frequency bands also showed clear age-related findings with specific topographical differences. Globally power increased with age in both the delta and theta band. Delta and theta power were significant and more distinct around frontal regions. In Figure 1c, both age groups showed a clear increase of power in frontal areas and an occipital maximum for delta power. Analysis of the averaged spectrograms for the two age groups showed the theta peak becomes faster and more pronounced with age (Figure 1b). Thus, the older group had a slightly faster theta peak. Similar to sleep spindles, not all participants showed a distinct theta peak, thus individual level analysis could not be performed. Power in the theta band also showed a significant increase for frontal locations. Beta activity showed a significant decrease with age, predominately in posterior temporal regions. However, after non-parametric suprathreshold cluster analysis, the cluster sizes of significant electrodes did not remain significant.

Research question 1b. What physiological activity patterns of NREM sleep are associated with development in infants/toddlers 12-30 months of age?

To examine the physiological patterns of NREM sleep and associations with development, descriptive data examining performance on the MSEL and the VABS are shown below (Table 5). Table 5 shows means, SD, and p values for the older and younger groups on the MSEL and VABS full composite. Table 5 also shows the domain standard scores on the MSEL for Visual Perception, Fine Motor, Receptive and Expressive Language and the VABS Communication, Daily Living, Socialization, and Motor. First, performance and correlations with the MSEL are presented, followed by performance and correlations with the VABS.

Table 5. Distribution of Performance

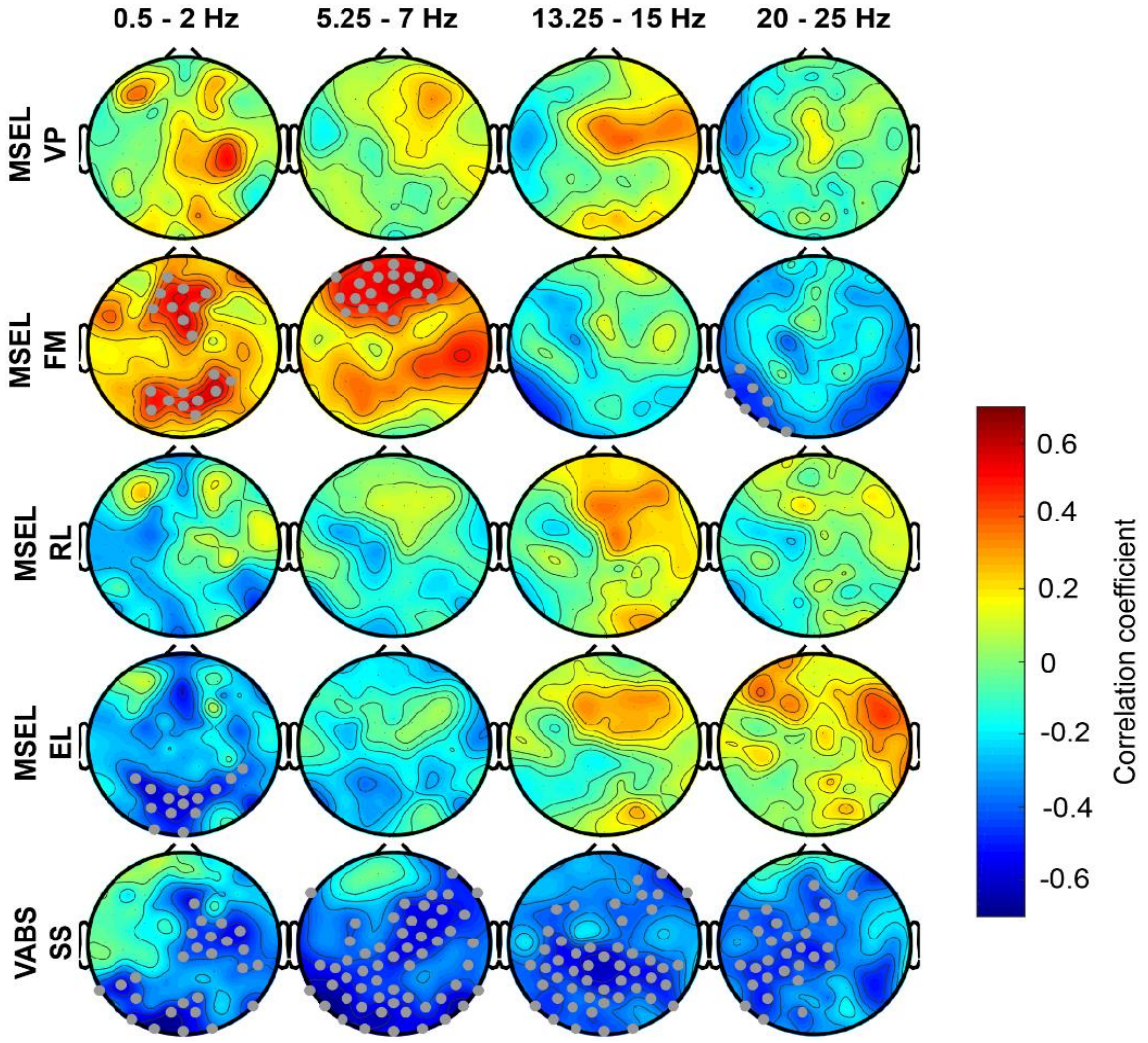
	Younger group (n=14)		Older group (n=13)		
	Mean	(SD)	Mean	(SD)	P value
MSEL	98.36	9.09	105.54	12.19	0.099‡
MSEL VP	48.21	7.76	48.69	8.10	0.877
MSEL FM	54.43	6.55	49.46	9.29	0.125
MSEL RL	42.93	7.09	52.15	9.97	0.011*
MSEL EL	50.07	7.29	60.31	11.71	0.014*
VABS	94.43	11.62	102.31	9.60	0.066‡
VABS Communication	94.93	13.28	107.31	8.36	0.024*
VABS Daily Living	96.00	13.49	99.77	11.21	0.693
VABS Socialization	95.00	5.36	101.08	8.81	0.045*
VABS Motor	95.29	11.09	100.69	8.63	0.238

Trend level P value < 0.1‡, significant P value < 0.05*
MSEL-Mullen Scales of Early Learning Composite
VP=Visual Perception
FM=Fine Motor
RL=Receptive Language
EL=Expressive Language
VABS-Vineland Adaptive Behavior Scales- Second Edition composite
VABS Communication= Communication domain
VABS Daily Living= Daily Living domain
VABS Social Domain= Social domain
VABS Motor Domain= Motor domain

MSEL. Though the MSEL is norm referenced and controls for age, both Receptive (Younger: *M*= 42.93, *SD*= 7.09; Older: *M*= 52.15, *SD*= 9.97) and Expressive Language (Younger: *M*= 50.07, *SD*= 7.29; Older: *M*= 60.31, *SD*= 11.71) on the MSEL showed significant differences between the younger and older groups (Table 5). In this sample, Expressive and Receptive Language (r=0.47, p=0.01) are highly associated with overall performance on the MSEL (see Table 6). Overall performance on the MSEL trends towards significant, and as one would expect during this specific time period, the older group will likely have more exposure with language and likely increased communicative skillsets. Thus, more experience with language may be accounting for the observed difference.

Given the frequency bands that were found to change with age (findings from research question 1a), it was next examined if these same frequencies were related to performance on the MSEL. Specifically, delta, high theta, middle sigma (the spindle band), and beta were assessed for possible relations with performance. Figure 3 shows computed partial correlations controlled for age. Though the overall composite of the MSEL was not correlated with age, some domain scores on the MSEL were associated with age and specific frequencies. One possible reason for this finding is due to the modest sample size and a larger sample would yield specific correlations with the MSEL composite. Another possible reason for these findings are specific frequencies are associated with specific skillsets. For example, Fine motor skills (measured by the MSEL, Table 6) were positively correlated with low delta (.5-2 Hz) at frontal and posterior regions and high theta (5.25-7 Hz) in frontal electrode sites. Fine motor skills were negatively correlated with beta (20-25 Hz) at parietal electrode sites. Whereas Expressive Language showed significant negative correlations over frontal and occipito-temporal regions in the delta band. Neither Visual Perception and Receptive Language showed any significant correlations. Thus, it may be the case that more sophisticated or higher order skillsets as Expressive Language are negatively correlated with lower frequencies while more basic skillsets as Fine Motor are positively correlated with lower frequencies.

Figure 3. Topographic Correlations with Performance



Topographical representation of Pearson correlation coefficients between different bands of spectral power, cognitive measures and domains. Electrodes that showed significant correlations after permutation statistical correction (SnPM) marked with grey dots. Mullen Scales of Early Learning with subdomains (MSEL: Visual Perception, Fine Motor, Receptive Language, and Expressive Language). Vineland Adaptive Behavior Scale Standard Score (VABS domains: Communication, Daily Living Skills, Socialization, and Motor Skills).[3]

VABS. Similar to the MSEL, both the Communication (Younger: *M*= 94.93, *SD*= 13.28; Older: *M*= 107.31, *SD*= 8.36) and Socialization (Younger: *M*= 95.00, *SD*= 5.76; Older: *M*=

[3] This is a pre-copyedited, author-produced version of an article accepted for publication in *Sleep* following peer review. The version of record (Page, Lustenberger, & Frohlich, 2018) is available online at: https://doi-org.libproxy.lib.unc.edu/10.1093/sleep/zsy024.

101.08, *SD*= 8.81) domain show significant differences between the younger and older groups (Table 5). Socialization (r=0.37, p=0.06) and overall performance (r=0.33, p=0.09) of the VABS trends towards significance (Table 6). Communication showed positive correlations with age. Given that communication and socialization are examining aspects of language, it is not surprising to see differences between the younger and older group.

EEG data that was found to change with age, specifically delta, high theta, sigma (spindle band), and beta, were assessed for possible relations with performance on the VABS. Figure 3 shows computed partial correlations controlled for age. The standard score of the VABS was negatively associated with power values in all of the examined frequency bands. Significant electrodes were widely distributed and not localized to a specific scalp location.

Table 6. Correlations with Age

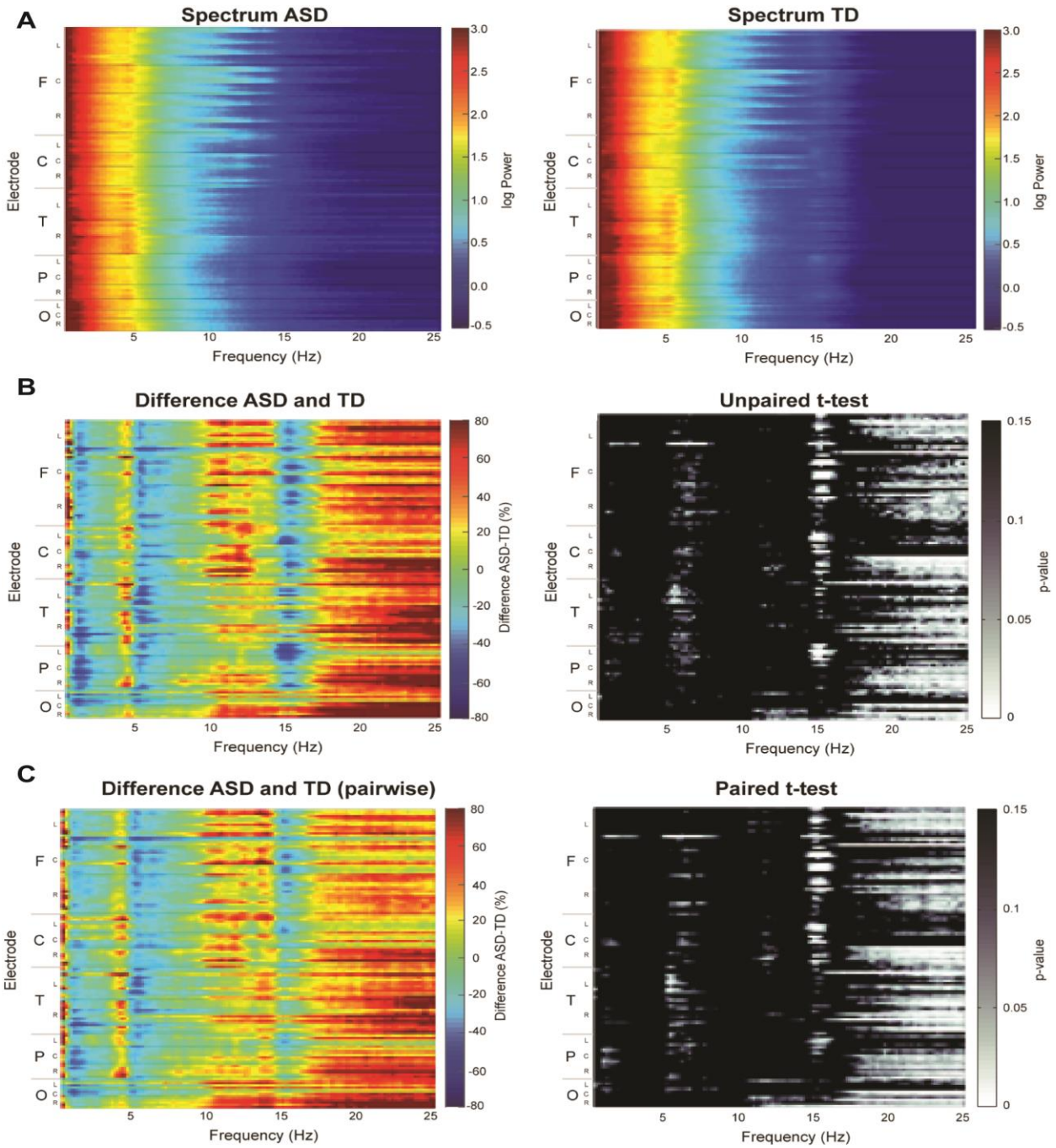
Correlation	Measure	R	P
Age	MSEL Composite	0.29	0.14
	MSEL VP	0.16	0.42
	MSEL FM	-0.35	0.07‡
	MSEL EL	0.47	0.01*
	MSEL RL	0.47	0.01*
	VABS Composite	0.33	0.09‡
	VABS Communication	0.49	0.01*
	VABS Daily Living	0.12	0.55
	VABS Socialization	0.37	0.06‡
	VABS Motor Domain	0.2	0.39

Trend level P value < 0.1‡, significant P value < 0.05*
MSEL-Mullen Scales of Early Learning Composite
Visual=Visual Perception
FM=Fine Motor
EL=Expressive Language
RL=Receptive Language
VABS =Vineland Adaptive Behavior Scales- Second Edition
VABS Communication= Communication domain
VABS Daily Living= Daily Living domain
VABS Social Domain= Social domain
VABS Motor Domain= Motor domain

Research question 2a. Given the physiological activity patterns of NREM sleep in infants/toddlers, what patterns of NREM sleep are associated with ASD?

To investigate the physiological patterns and associations of NREM sleep in ASD, participants were matched on age and then grouped into the ASD and TD group. To address research question 2 (a and b) this study used primary and secondary analyses. Since the results of both the primary and secondary analyses are very similar, tables and figures for the primary analyses are provided below, and all tables and figures for the secondary analyses are located in Appendix E, F, G, H, and I. Both primary and secondary analyses showed physiological differences are clearly present in both the main features of NREM sleep and other investigated frequencies. Below, figure 4 provides a visual description of the findings from the primary analysis and the differences with spectral power between ASD and TD. The top row (A) shows spectral power for ASD (left) and TD (right). The second (B) and third (C) rows, left column, show correlation heat maps of the differences between ASD and TD. The second (B) and third (C) rows, right column, shows unpaired and paired t tests between ASD and TD.

Figure 4: Spectral Power in ASD and TD

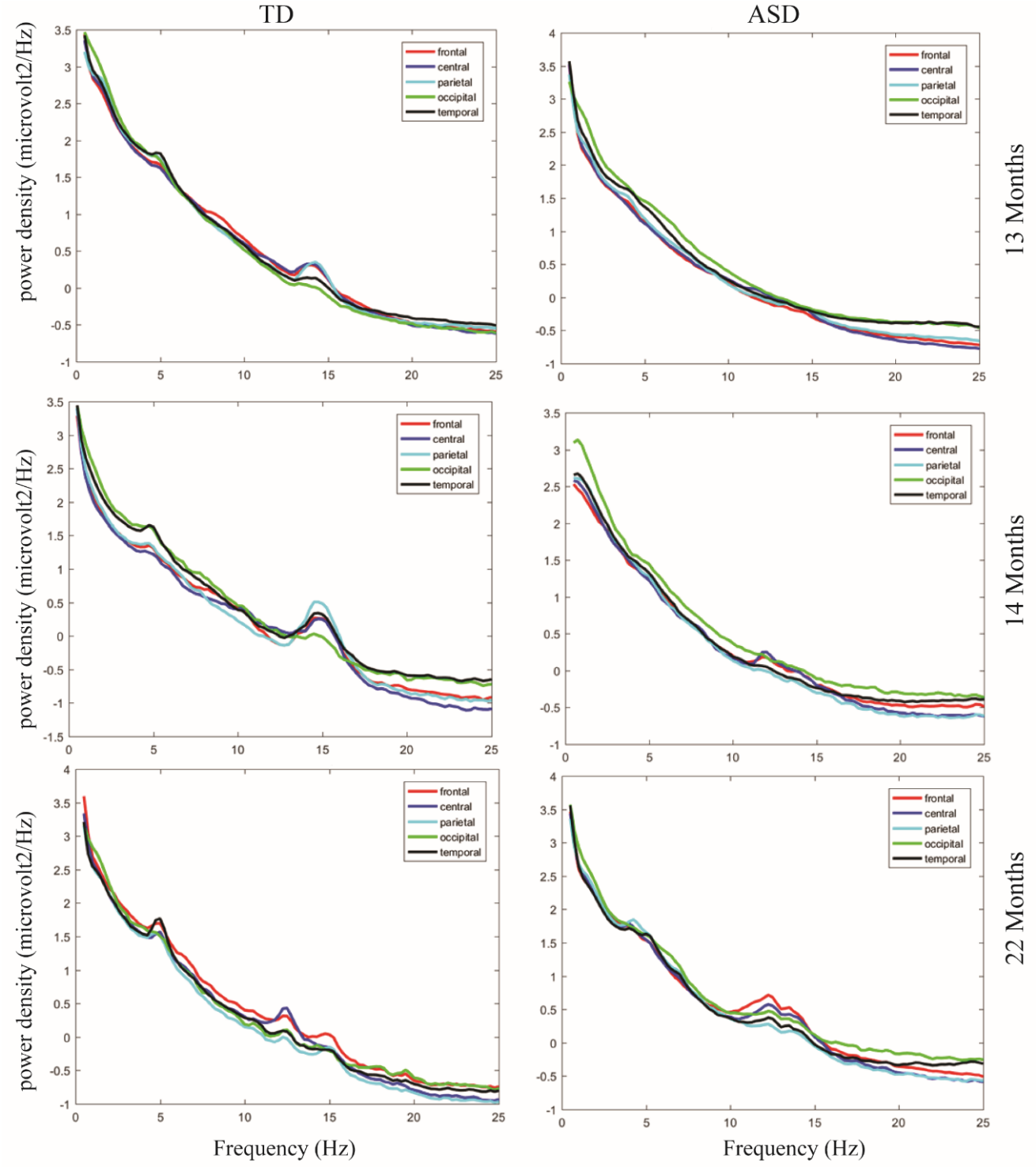


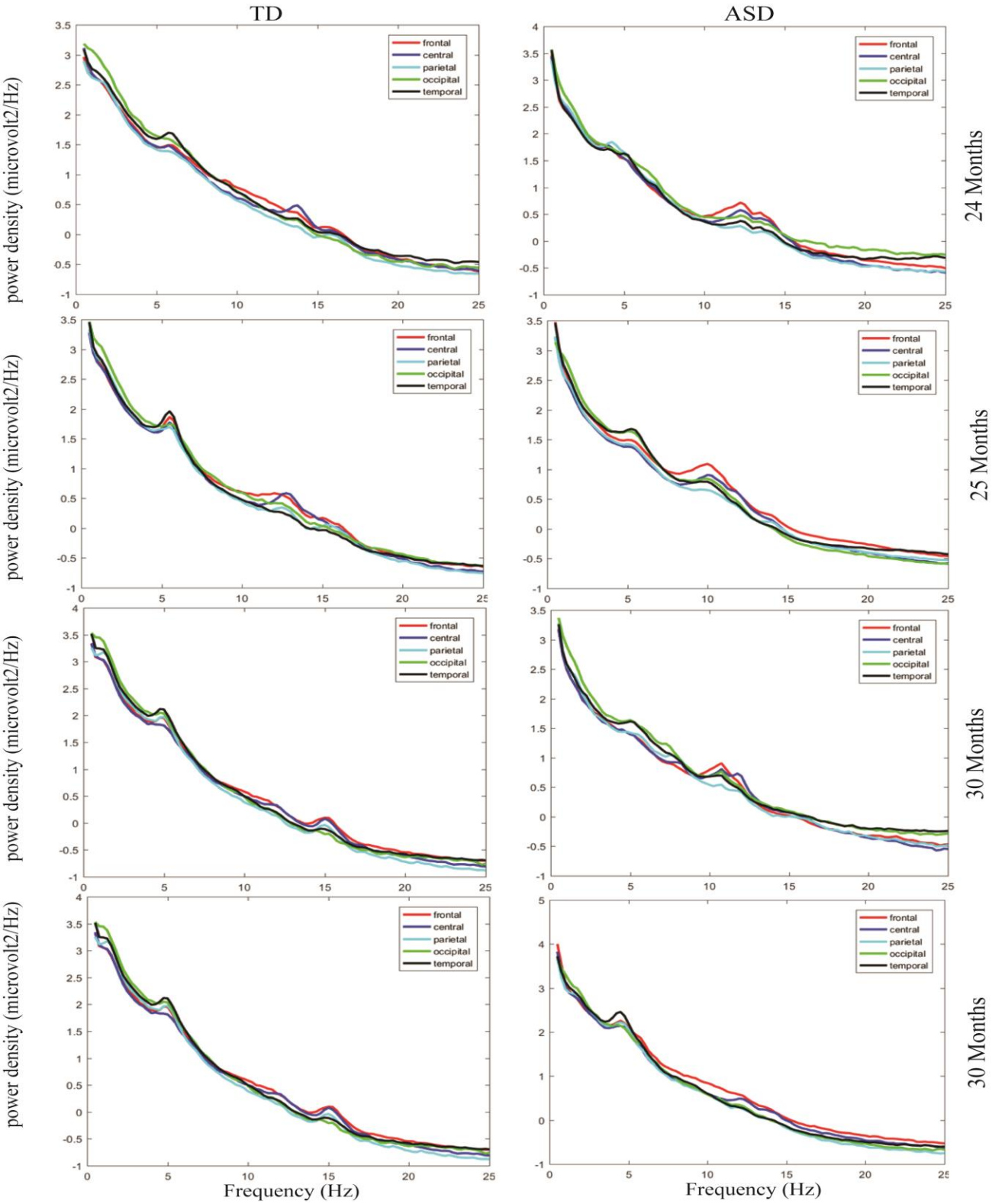
(A) Spectral power across all participants for ASD (first row, left) and typically developing (TD) comparison (first row, right) (B) Heat map of the difference of NREM spectral power values during NREM in ASD and TD. Left: Differences in spectra power are depicted with warm and cold colors, respectively. Second Row Right: unpaired t-test. (C) Heat map of the difference of NREM spectral power values during NREM in ASD and TD (pairwise). Left: Difference in spectra power are depicted with warm and cold colors, respectively. Third Row Right: Paired t-test.

Sleep Spindles. Figure 4 highlights the statistical differences of the spindle band detected with the primary analysis. Investigation of the spectrogram for the group with ASD showed positive correlations were found for middle spindle frequencies (11.50–13 Hz). These higher middle frequencies were localized to primarily frontal electrode sites, whereas TD showed power values in the slow and fast spindle range in central, parietal, and temporal cites. These findings were very similar to the secondary analysis (see, Appendix E) also showing correlations with slow and fast spindles. Differences in detecting all spindle peak properties (as shown in figure 2 in findings from 1a) could not be quantified because not all participants showed a clear spindle peak, and in some instances no spindle peak was observed.

The increased middle spindle power in ASD may be best explained by differences in spindle density. Figure 5 depicts individual spectral density plots for aged matched participants from the primary analysis. Participants with ASD either have a slower spindle (10-12 Hz) peak (in between the slow and fast, middle spindle) or in some cases (for example at 13 and 30 months) no peak. In contrast, many of the age-matched TD have a pronounced slow and fast peak.

Figure 5: Spectral Density of Individual Participants





Spectral density for individual participants. Participants with ASD (left, column) and selected age matched TD (right, column). Electrode sites are denoted as follows: Frontal (red), central (dark blue), parietal (light blue), occipital (green), and temporal (black).

SWA. The other prominent feature of NREM sleep, SWA was also examined for group and individual differences. Across all participants, nap recordings were primarily composed of NREM sleep with similar amount of Stage 2 and Stage 3 sleep. Delta activity, an indicator of SWA that is dominant during Stage 2 and 3 was examined. Delta was similar across groups at both the group and individual level, and thus, differences in SWA were not observed. Figure 4 (primary analysis) and Appendix E (secondary analysis) reiterates this finding, with neutral colors in the heat map (left) and no statistical differences in spectral power (right).

NREM findings. Other investigated frequencies showed significant differences between ASD and TD. One of the main differences between ASD and TD, was power spectral density in the fast theta (5.25-7 Hz) band. Investigation of the spectra for the two groups revealed that the theta peak became slightly faster and more pronounced in the TD group (Figure 4). Figure 4 (primary analysis) and Appendix E (secondary analysis) further illustrates the TD group with increased theta in frontal, central and parietal electrode sites compared to ASD. The difference in theta is best explained by visual inspection of the individual spectral density plots (Figure 5). At an individual level, participants with ASD either have reduced or no prominent theta peak. In comparison, all participants in the TD group showed a prominent faster theta peak.

One of the most obvious differences in the spectral features was in the beta band. Higher beta (20-25 Hz) frequencies were clearly widespread in ASD. Differences in beta were detected in frontal, central-parietal, temporal, and occipital areas. Secondary analyses (Appendix E), also showed widespread differences in beta, with significantly increased beta in ASD. At an individual level (Figure 5), 6 out of 7 participants with ASD showed increased beta (tail end of the plot).

Research question 2b. Given the physiological activity patterns of NREM sleep and associations with development in infants/toddlers, what features of NREM sleep are associated with development in infants/toddlers with ASD?

Based on the frequencies (patterns) of NREM sleep that were shown to differentiate TD and ASD (theta, sleep spindles, and beta), were examined for associations with development in ASD. From the primary analysis, Table 7 provides descriptive statistics examining performance on the MSEL and the VABS. Means, SD, and p values (derived from the Mann-Whitney U) for ASD and TD for the full composite and domains on the MSEL (Visual Perception, Fine Motor, Receptive and Expressive Language) and VABS (Communication, Daily Living, Socialization, and Motor) are presented. Despite the small sample, significant differences were found between groups. These differences are further supported by the results of the secondary analyses in Appendix F. Findings are presented in order of assessment. First, performance and topographic correlations with the MSEL, followed by performance and topographic correlations with the VABS, and correlations with autism severity.

Table 7. ASD and TD Distribution of Performance

	TD group (n=7)		ASD group (n=7)		
	Mean	(SD)	Mean	(SD)	P value
MSEL	100.14	15.56	73.86	16.35	0.001**
MSEL VP	46.00	6.06	40.14	7.95	0.021*
MSEL FM	51.29	7.78	38.71	15.15	0.068‡
MSEL RL	48.14	11.54	31.43	10.64	0.007*
MSEL EL	51.86	13.22	32.86	12.63	0.023*
VABS	89.86	12.58	79.71	10.87	0.120
VABS Communication	94.57	14.88	79.86	14.85	0.053‡
VABS Daily Living	87.29	13.38	73.57	18.15	0.031*
VABS Socialization	92.71	9.48	81.43	12.00	0.025*
VABS Motor	92.71	8.22	90.29	9.78	0.676

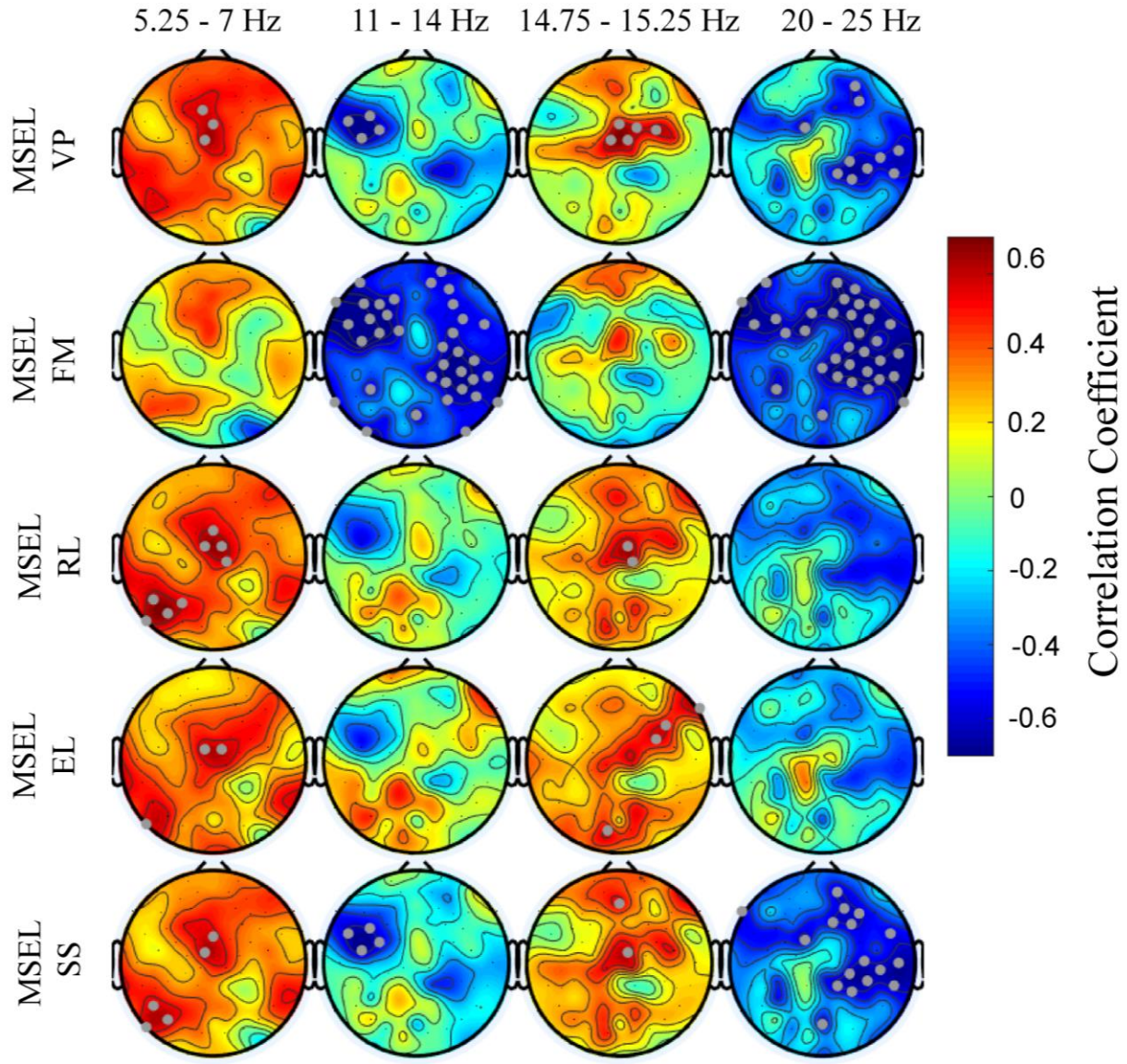
Trend level P value < 0.10‡, significant p value <0.05*, p value < 0.005**
MSEL-Mullen Scales of Early Learning Composite
VP=Visual Perception

FM=Fine Motor
RL=Receptive Language
EL=Expressive Language
VABS-Vineland Adaptive Behavior Scales- Second Edition composite
VABS Communication= Communication domain
VABS Daily Living= Daily Living domain
VABS Social Domain= Social domain
VABS Motor Domain= Motor domain

MSEL. Across many domains on the MSEL, there were clear statistical group differences. Receptive (ASD: *M*= 31.43, *SD*= 10.64; TD: *M*= 48.14, *SD*= 11.54 and Expressive Language (ASD: *M*= 32.86.44, *SD*= 12.63; TD: *M*= 51.86, *SD*= 13.22) and overall performance were significantly different between groups. Given that one defining feature of ASD are delays or impairments in social communication, the significant differences in both Expressive and Receptive Language between ASD and TD are expected. Visual Perception also, showed significant group differences (ASD: *M*= 40.14, *SD*= 7.95; TD: *M*= 46.00, *SD*= 6.06). Only Fine Motor showed trend level differences (ASD: *M*= 38.71, *SD*= 15.15; TD: *M*= 51.29, *SD*= 7.78). Secondary analyses (Appendix F) showed that with an increased sample, all domains except fine motor showing trend level differences (p =.068) remained statistically significant. The results with the primary and secondary analyses were almost identical and this is likely due to the non-independent nature of the resampling technique. Thus, an increased sample of different aged matched participants (not resampled) could be used to further distinguish group performance.

Given the statistical differences with performance in ASD and TD, it was next examined if the domain and standard scores were correlated with the NREM features (differences in power) that differentiated the two groups. To this, all participant's (ASD and TD) performance on the MSEL was correlated with fast theta (5.25-7 Hz), middle spindles (11-14 Hz) and fast spindles (14.75-15.25 Hz), and beta (20-25 Hz) frequencies (Figure 6).

Figure 6 Topographic Correlations: MSEL with ASD and Matched TD



Topographical representation of Pearson correlation coefficients between different bands of spectral power, MSEL measures and domains. Mullen Scales of Early Learning with subdomains (MSEL: Visual Perception, Fine Motor, Receptive Language, and Expressive Language, and the MSEL standard score for the overall composite). Electrodes that showed significant correlations after permutation statistical correction (SnPM) marked with grey dots.

Findings from the primary analysis revealed Visual Perception was positively correlated with fast theta and a cluster of central electrodes in the fast spindle band. Fine Motor showed

trend level correlations with fast theta which mirrors the trend level findings in Table 7. The secondary analyses showed both Visual Perception and Fine Motor were positively correlated with fast theta and a cluster of central electrodes in the fast spindle band (Appendix G). Visual Perception showed left frontal negative correlations with middle spindles and widespread significant negative correlations with beta. Fine Motor also showed widespread negative correlations with both middle spindles and beta activity. The findings from both analyses, suggest participants with higher performance on Fine Motor and Visual Perception had increased theta and fast spindles while participants with lower performance had increased middle spindles and beta activity.

Both primary and secondary analyses with Receptive Language revealed significant positive correlations with fast theta and frontal spindle regions and showed trending negative correlations with beta frequencies. The secondary analyses provide support that an increased sample yields additional significant findings. Specifically, negative correlations were found in left frontal electrode cites in the middle spindle band and centro-parietal electrode cites in beta frequencies (Appendix G).

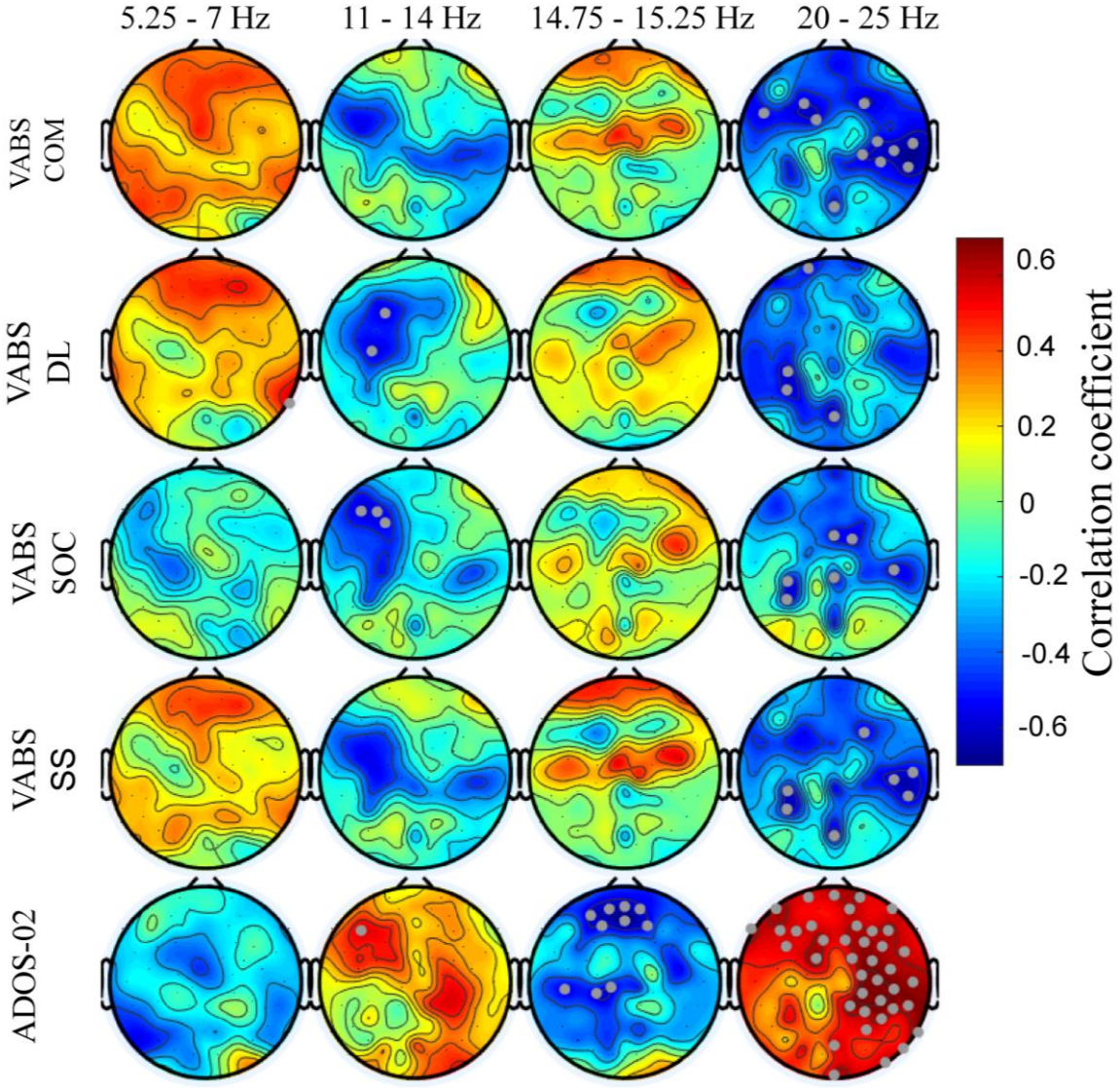
The primary analyses with Expressive Language showed modest significant positive correlations in central electrode sites with fast theta and a right cluster of electrodes in fast spindles. The secondary analyses exhibited slightly different results with trend level correlations with fast theta and fast spindles and a modest significant negative correlation over a temporal cite in beta. The MSEL Standard Score, shows that overall performance is positively correlated with a cluster of electrodes in fast theta and fast sleep spindles, while middle spindles and beta were negatively correlated with performance on the MSEL. Secondary analyses (Appendix G), further illustrates these findings with overall performance on the MSEL. In other words,

participants with higher performance on the MSEL had increased fast theta and fast spindles and reduced middle spindles and beta frequencies.

VABS. Despite the small sample size and after controlling for multiple comparisons, Table 7 shows clear group differences across various domains on the VABS. The primary analyses revealed Daily Living and Socialization domains are significantly different between groups, while Communication shows trend level differences between groups. The Motor domain was not statistically different between groups. Motor is composed of two subdomains, Fine and Gross Motor. With the addition of Gross Motor and no known motor impairments in this sample, this could account for this finding. With a larger sample, the secondary analysis showed Socialization, Daily Living, and Communication to be significantly different and the overall composite near trend level significance (Appendix H).

Primary and secondary analyses were conducted to assess possible correlations with domain and standard scores and NREM features that differentiated the two groups. Participant's (ASD and TD) performance on the VABS (Communication, Daily Living, Socialization, Standard Score) was correlated with fast theta (5.25-7 Hz), middle (11-14 Hz) and fast spindles (14.75-15.25 Hz), and beta (20-25 Hz). Figure 7 illustrates the findings from the primary analyses of the topography of correlations with the VABS.

Figure 7 Topographic Correlations: VABS and ADOS-2 with ASD and Matched TD



Topographical representation of Pearson correlation coefficients between different bands of spectral power, cognitive measures and domains (corrected for age). Electrodes that showed significant correlations after permutation statistical correction (SnPM) marked with grey dots. Vineland Adaptive Behavior Scale standard score (VABS: Communication, Daily Living Skills, Socialization, and VABS composite) and the Autism Diagnostic Observation Schedule (ADOS-02).

The primary analysis revealed trend level group differences with Communication and fast theta and fast spindles. Except for one significant electrode cite with Daily Living, both Daily Living and Socialization also showed trend level differences in fast theta and the fast spindle band. Interestingly, the secondary analysis showed no trend level differences in Communication,

Daily Living, and Socialization. One reason for the observed difference between the primary and secondary analyses are due to the resampling of participants. A larger sample with different age matched participants (not resampled) would allow for a clearer depiction of NREM associations in Communication, Daily Living, and Socialization.

Both primary and secondary analyses revealed the middle spindle band was negatively correlated with Socialization and Daily Living in left fronto-central regions. The clearest finding is widespread negative correlations of beta, and all domains and overall performance on the VABS. The secondary analyses showed similar and more robust correlations (Appendix H). The middle spindle band was negatively correlated with Communication in right parieto-occipital regions, Daily Living in frontal central regions, and a few electrodes sites in Socialization, and the VABS Standard Score. Again, beta showed widespread negative correlations with all domains on the VABS.

Overall, outcomes from the primary and secondary analyses suggests the findings VABS are similar to the results on the MSEL. Findings revealed trend level to significant differences in fast theta and sleep spindles, while slow spindles and beta were negatively correlated with performance on the VABS. In other words, participants with higher performance on the VABS showed increased fast theta and fast sleep spindles and reduced middle spindles and beta frequencies. Whereas participants with lower scores on the VABS had increased middle spindles and beta activity. Appendix H highlights the findings of the secondary analyses which suggest with an increased sample size, these findings persist and further exhibit pronounced differences with middle spindles and beta frequencies in ASD and TD.

ADOS-2. Autism severity was examined in regards to participants performance on the MSEL and VABS. Primary and secondary analyses used Pearson correlation coefficients to

examine correlations with autism severity. Table 8 depicts the results from the primary analyses. Despite the small sample scores on the ADOS-02 were negatively correlated with many domain scores and overall performance on the MSEL and VABS. Secondary analyses also showed widespread negative correlations (Appendix I). In other words, participants with high scores on the ADOS-2 or rather increased symptoms of autism showed lower overall performance on the MSEL and VABS.

Table 8. Correlations with ADOS-2

Correlation	Measure	R	P
ADOS-2	MSEL Composite	-0.74	0.003*
	MSEL VP	-0.48	0.085‡
	MSEL FM	-0.59	0.028*
	MSEL EL	-0.68	0.007*
	MSEL RL	-0.72	0.004*
	VABS Composite	-0.52	0.057‡
	VABS Communication	-0.57	0.034*
	VABS Daily Living	-0.41	0.148
	VABS Socialization	-0.60	0.024*
	VABS Motor Domain	-0.24	0.408

Trend level P value < 0.10‡, significant p value <0.05*, p value < 0.005**

MSEL-Mullen Scales of Early Learning Composite
VP=Visual Perception
FM=Fine Motor
EL=Expressive Language
RL=Receptive Language
VABS =Vineland Adaptive Behavior Scales- Second Edition
VABS Communication= Communication domain
VABS Daily Living= Daily Living domain
VABS Socialization Domain= Social domain
VABS Motor Domain= Motor domain

All domains on the MSEL and overall composite were negatively associated with symptom severity. Only Visual Perception (MSEL) showed trend level negative correlations. Communication and Socialization (VABS) were negatively correlated with symptom severity. Since difficulties in social communication are a defining feature of ASD, the negative

correlations in Language and Socialization are expected. Only Daily Living and Motor domains on the VABS were not correlated with symptom severity. Findings from the secondary analysis (Appendix I) suggest with a larger sample, these differences not only persist, but show increased differences between the groups, where Visual Perception (MSEL) and Daily Living (VABS) exhibited significant group differences.

To further examine severity of autism and features of NREM sleep, participant's (ASD and TD) performance on the ADOS-2 was correlated with fast theta (5.25-7 Hz), middle (11-14 Hz) and fast spindles (14.75-15.25 Hz), and beta (20-25 Hz). Figure 7, bottom row illustrates the correlations of autism severity from the primary analysis. The bottom row shows that frontal and central electrodes sites are negatively correlated with fast spindles. The secondary analysis (Appendix H) yields similar findings with some electrode sites are negatively correlated with left temporal regions with fast theta and sleep spindles. More simply put, participants with low performance on the ADOS-2 had significantly increased fast theta and sleep spindles. Primary and secondary analyses showed the most consistent findings, are widespread positive correlations with beta and the ADOS-2 and a small electrode site in the middle spindle band. As expected, participants who scored high on the ADOS-2 have significantly more beta than participants who scored low on the ADOS-2. In other words, participants with ASD showed increased beta activity.

Summary

In summary, the NREM characteristics reflected in the sleep EEGs showed clear developmental changes with age in delta (.5-2 Hz), theta (4-7 Hz), sleep spindle band (10-16Hz) and beta (20-25 Hz) oscillations in the 12-30-month age span. Infants/toddlers who were 12-20 months had a slower theta peak (5.25 Hz) and showed a prominent spindle peak at 14 Hz

whereas infant/toddlers between 20-30 months had a faster theta peak (7 Hz) and a prominent slow and fast spindle peak (11.75 and 15 Hz, respectively). Many of these frequencies were highly correlated with a number of domains on the MSEL and VABS, particularly Fine Motor, Language, and Socialization in the 12-30-month age range.

Primary and secondary analyses exhibited statistically different NREM sleep patterns in ASD and TD. In ASD there was decreased power and density in fast theta and sleep spindles, and increased power and density in the middle spindle and beta frequencies. At an individual level, these findings were also present. In most cases, individuals with ASD did not have a slow and fast spindle peak. Increased beta activity was a defining feature between the groups and this was also seen at the individual level. Topographic features also showed vastly different correlations with performance on the MSEL and VABS between ASD and TD. Middle spindle and beta band showed widespread negative correlations in ASD.

Finally, autism severity (ADOS-2) was negatively correlated with performance on the MSEL and VABS. In other words, participants with high scores on the ADOS-2 or rather increased symptoms of autism showed decreased performance on the MSEL and VABS. In contrast, participants with low scores on the ADOS-2 had higher scores on the MSEL and VABS. The secondary analysis provides support for these findings and the benefits of a larger sample which allowed for a clearer distinction between groups.

Overall, findings from question 1 (a and b) showed NREM features exhibit maturational differences between the younger (<20 months) and older (>20 months) group that are associated with various skillsets (Language, Fine Motor, and Socialization). Question 2 (a and b) revealed clear differences in features of NREM sleep were detected between TD and ASD. Irrespective of the small comparative sample for research 2 (a and b) primary and secondary analyses showed

features of NREM sleep were highly associated with language and communication skillsets. Impairments in language and communication are a defining feature of ASD and this was clearly reflected in the associated features of NREM sleep.

CHAPTER FIVE: DISCUSSION

The goals of this study were to examine: (1a) The physiological activity patterns of NREM sleep (sleep spindles and SWA) in infants/toddlers 12-30 months of age, (1b) the physiological activity patterns of NREM sleep associated with development in infants/toddlers 12-30 months of age, (2a) the physiological activity patterns of NREM sleep that are associated with infants/toddlers with ASD, and (2b) the physiological activity patterns of NREM sleep and associations with development in infants/toddlers with ASD. To address the research questions, developmental data collected during a home visit was compared with hdEEG nap recordings during a lab visit. The findings illustrated age related changes in delta (.5-2 Hz), theta (4-7 Hz), sleep spindle band (10-16Hz) and beta (20-25 Hz) oscillations. These NREM frequencies were highly correlated with domain scores of the Fine Motor and Expressive Language scales on the MSEL and the overall composite on the VABS. NREM findings at both the group and individual level showed significant differences between infants and toddlers with ASD and those who were TD. For the infants/toddlers with ASD, these NREM features were negatively correlated with performance on the MSEL and VABS, while autism severity (ADOS-2) showed a strong positive correlation with beta activity. These findings suggest an important role of NREM sleep in cortical maturation and the associated development of cognitive and social behavioral skillsets during this important developmental period. These findings also provide support for the role of NREM sleep as a potential biomarker to distinguish typical development.

This chapter is organized by research question and examines the results with regard to prior research findings and the implications for future research. First, research question 1a and 1b will be discussed with a summary of the results. Then, research question 2a and 2b will be discussed with a summary of the results based on the primary and secondary analysis. This is followed by an examination of how the results of this study relate to and extend the existing research on NREM sleep and risk for ASD. Next, the limitations of the study are examined. Then, considerations and directions for future research are discussed, followed by the study's conclusions.

Characterizing NREM Sleep in Infants/Toddlers

The main findings indicated age related changes in power in low delta, high theta, and beta frequency bands, and in sleep spindle activity. These changes include age related changes in power and topography in SWA, sleep spindles and various EEG features. Findings for sleep spindles are first discussed, followed by SWA, and EEG features at other frequencies present during NREM. Then, an examination of these NREM findings and associations with various developmental domains are discussed.

Sleep Spindles. The study demonstrated a change in the spindle frequency band that was characterized by decreased power in posterior regions. The reduction in spindle power appears to be explained by a decrease in density (number/min) and duration of sleep spindles in the 12-30-month age span. This finding is consistent with previous research showing a significant decrease in spindle frequency at 2-5-years of age (Kurth et al., 2010) and extends the literature by showing that the observed decrease occurs earlier in development. This decrease in posterior regions reflects a shift from a single spindle peak in younger children (12-20 months) (around 14 Hz) to a double peak (around 12 and 16 Hz) with a trough around 14 Hz in older children (20-30

months). These findings suggest that the presence of the double (slow and fast) spindle comes about around 20 months of age. Previous research has indicated the formation of the double spindle at 24 months (Jankel & Niedermeyer, 1985), however, based on this study's results this process appears to occur earlier in development around 20 months of age.

Alterations of sleep spindle characteristics are thought to mirror maturation of the CNS (Andrillon et al., 2011; Tanguay et al., 1975) and the overall plasticity of early brain development. In the older group (>20 months), fast sleep spindles appear to be "ultrafast" (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017) and possibly a characteristic of toddlerhood. In later development around 5 years, these "ultrafast" frequencies disappear and emerge to appear more like the fast spindle activity observed in adults (Kurth et al., 2010; Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017).

Sleep spindles also showed interindividual differences. Visual inspection of parietal spectra for each participant showed that only 2 of 15 in the younger group had a clear double spindle and those participants were 18 and 19 months, respectively. In contrast, all but one of the participants in the older group exhibited a double spindle peak. Similar to adults, large interindividual differences were observed (De Gennaro & Ferrara, 2003). Despite some consistent differences in the 12-30-month age span, developmental changes in NREM oscillations depict immense change in this age span. Research in older children have shown that these spindle changes are not consistent across later childhood and show rapid change well into puberty (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017).

SWA. A distinct finding in this study was the progression of SWA. SWA showed an increase with significant positive correlations of power in low delta (.5-2 Hz), high theta (5.25-7 Hz), sigma (spindle band, 11- 16 Hz), and beta (20-25 Hz) frequency bands with age. Prior

research by Kurth et al. (2010) showed maximal SWA activity progresses along the posterior-anterior axis across childhood through puberty (Feinberg, 1982; Gaudreau et al., 2001; Jenni et al., 2004; Campbell and Feinberg, 2009). The present study extends these findings and showed this trajectory in SWA is also present in the 12-30-months age span. Novelli et al. (2016) examined NREM features from birth to 48-months. While they did not specifically examine SWA, findings showed that delta activity (prominent rhythm of SWA) was primarily stable in posterior occipital regions. They also observed a shift of delta activity along the posterior-anterior axis which was positively correlated with age. Thus, the shift of delta activity that was observed by Novelli and colleagues, suggests the shift in SWA likely begins before 12 months of age. During early development, the observed increase of SWA is thought to reflect an increase in synapses and plasticity (Campbell & Feinberg, 2009; Kurth et al., 2010) in which the brain undergoes reorganization (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017). The reorganization of the brain likely reflects the processing of new information that is gained throughout the day, which is then retained during SWA in NREM sleep (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017).

Other Frequencies During NREM. The present study aimed to characterize the two main features of NREM sleep, sleep spindles and SWA. Other NREM findings were present in the 12-30-month age span. Similar to the research findings of Novelli et al. (2016), delta showed a frontal increase and an occipital maximum in power for both age groups. Power globally increased with age and was most pronounced over frontal regions. Given that Novelli and colleagues also found an increase in delta in a sample of 39 participants from birth to 48-months (mean age 15 months), the shift in delta power likely starts before the 12-30-month span examined in the present study. Findings in delta activity from the current study mirror the

findings with SWA, and extend prior research showing a developmental increase of delta activity across early childhood.

Power in the theta band also showed a significant increase for frontal locations and a trend for a right temporal lobe with age. Research in infants younger than 6 months, shows theta power increasing over the first few months of life (Chu et al, 2011). Longitudinal research with children at 2, 3, and 5 years also shows increasing theta power (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017). In contrast, adolescents show a decrease in theta power (Kurth et al., 2010) by adulthood, theta activity is no longer present during NREM sleep (Campbell & Feinberg, 2009). Thus, this increase in frontal theta suggests theta oscillations are unique to early childhood (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017) and reduce during the course of later development. Since sleep is relatively understudied in the beginning years of life, this may be a key feature of NREM sleep in early childhood and more research is needed to understand the role of theta during this period in development.

This is the first study detailing the presence of beta activity during NREM sleep in early childhood and the significant decrease of beta activity with age. Beta decreased over posterior and temporal regions, however the cluster sizes of significant electrodes did not remain significant after non-parametric suprathreshold cluster analysis. One explanation could be due to movement during sleep that introduced a myogenic artifact, muscle movement that contaminated the EEG or high impedance from movement of electrodes and either types of movement would be captured in higher frequencies as beta or gamma (Gabsteiger, Leutheuser, Reis, Lochmann, & Eskofier, 2014). Another possible reason is this is the first study using hdEEG allowing for increased spatial measurement in which the dense electrode montage covers more muscles than sparse electrodes, and therefore it’s possible that more muscle movement is being picked up.

This is also the first study characterizing the transition across infancy to toddlerhood, and the decrease in beta could be a characteristic of this age span.

NREM Associations with Infant/Toddler Development

A novel aspect of this study was examining development through the lens of sleep, specifically NREM sleep and associations at the behavioral level. This is the first study to examine NREM sleep and behavioral performance with traditional measures of child development in the 12-30-month age span. Domain scores and overall standard score on the MSEL and the VABS were compared with NREM frequencies shown to change with age: specifically, theta, delta, sigma (sleep spindle), and beta bands.

MSEL. Various frequencies were highly associated with multiple skillsets on the MSEL. Fine Motor skills were positively associated with low delta (.5-2 Hz) and high theta (5.25-7 Hz) activity at frontal and posterior electrode sites, and beta (20-25 Hz) activity at parietal sites. Though no prior research has exclusively examined Fine Motor and NREM, previous research in school aged children showed significant findings with motor activity and sleep spindles. In school aged children both Chatburn et al. (2013) and Astill et al. (2014) documented the density of fast spindles was positively associated with sensorimotor functioning (Chatburn et al., 2013) and faster response times on a motor task (Astill et al. (2014). The present study did not find correlations with sleep spindles and fine motor activity. One possible reason for this difference across studies is that sleep spindles are specifically correlated with more sophisticated forms of motor activity such as sensorimotor, motor learning, and procedural memory for motor skills (Fogel & Smith, 2011).

Expressive Language showed significant negative correlations over frontal and occipito-temporal regions in the delta band, whereas both Receptive Language and Visual Perception did not show any significant correlations with features of NREM sleep. Many studies have yet to

examine the relationship between delta activity and language or verbal reasoning. Though no previous research showed correlations with delta activity and verbal reasoning or IQ (Bang, Khalilzadeh, Hämäläinen, Watanabea, & Sasaki, 2014), prior research with adults and school aged children also showed no correlations with sleep spindles and verbal IQ (Geiger et al., 2011). Assessments in older populations typically do not assess expressive or receptive language, instead verbal IQ is used and thus may account for the differences in research findings. It could be that expressive language and other higher-level skillsets are positively associated with delta activity, while lower-level skillsets are negatively associated with lower frequencies, though more research is needed to support this speculation. In general, more research is needed to understand the role of basic and fundamental skillsets that are representative of early development and their associated features with NREM sleep.

VABS. Findings from the VABS overall composite and its subdomains were negatively associated with power values in the sigma (sleep spindle band). In other words, participants with lower spindle frequencies performed better on all examined domains of the VABS. Although no other studies have exclusively examined VABS and associations with sleep spindles or other frequencies present during NREM sleep, consistent findings across studies in school aged children are negative correlations with sleep spindle frequency.

The current study extends previous findings in school age children by showing that sleep spindle frequency was negatively correlated with the VABS subdomains (Communication, Socialization, Motor) and overall performance on the VABS. Spindle frequency has been consistently shown to be negatively correlated with various aspects of IQ while other spindle characteristics such as density and amplitude have mixed findings. Geiger et al. (2011) observed spindle peak frequency was negatively correlated with full scale IQ (WISC-IV) in school aged

children. Chatburn et al. (2013) also obtained negative correlations between spindle frequency and performance on the Stanford-Binet nonverbal working memory task (and other neuropsychological tasks). Similar research by Gruber et al. (2013) also documented a similar negative association between spindle frequency and the WISC-IV on perceptual reasoning as well as working memory. Although these studies do not differentiate between findings with slow and fast spindles, collectively these findings suggest that young and school age children show negative correlations with sleep spindle frequencies on IQ, perceptual reasoning and working memory. In contrast, research with adults shows positive correlations of IQ with spindle characteristics as frequency and density (De Gennaro & Ferrara, 2003; Luthi et al., 2014). These findings suggest that sleep spindles may have a different function in child development and could be used as an indicator for the level of cognitive development (Gruber & Wise, 2016).

Characterizing NREM and Risk For ASD

This study sought to characterize NREM sleep for infants and toddlers who have or are at risk for ASD. Among populations with neurodevelopmental disorders, the influence of altered features of NREM sleep may be increased (Gruber & Wise, 2016) and thus, individuals with ASD, may be at increased risk of abnormal NREM characteristics. Interestingly, this was not the case with SWA as SWA was not statistically different between TD and ASD. However, other NREM findings were found. Despite the small age matched sample, both primary and secondary analyses revealed statistically different NREM features in infants with ASD. These NREM differences include decreased power and density in fast theta and sleep spindles and increased power and density in the middle spindle and beta frequencies, were present in the infants with ASD. Findings from both the primary and secondary analyses for sleep spindles are first discussed, followed by SWA, and other frequencies differentiating the two groups. Then, an

examination of ASD symptomatology and NREM findings and associations with various developmental domains are discussed.

Sleep Spindles. Excessive sleep spindle frequency in the middle spindle band was observed in ASD. Pioneering research by Gibbs and Gibbs (1962) reported "extreme spindles" with higher magnitudes and faster frequencies in children with intellectual disability (ID). Gibbs and Gibbs (1962) found fewer spindles over central and prefrontal regions, where fast and slow spindles occur. The present study lends credence to this finding as both primary and secondary analyses found the ASD group showed increased frequency for the middle spindle band and decreased frequencies and density in slow and fast spindles. At the individual level this finding was also consistent across participants with ASD. Topographic maps showed that in many instances the decreased frequencies observed in all participants with ASD appeared more like the youngest infants examined in research question 1 (a and b), and thus, at the neural level appear to look more like infants at 12 months or possibly younger.

As indicated in the literature review Tessier et al. (2015) is the only study to specifically examine sleep spindle characteristics (density) in school-aged children (mean=10.23 years) with high functioning autism (HFA). Findings showed the group with HFA had fewer fast spindles than the control group. This finding is consistent with the primary and secondary findings of the present study, in which participants in the ASD group had fewer fast spindles. Research by Bruni et al., (2007) did not specifically examine sleep spindles, but did find minor differences in the sleep patterns in NREM sleep. In that study, the groups with Asperger's and ASD showed decreased stage 2 sleep. Sleep spindles and more specifically fast spindles primarily occur during stage 2 sleep. Considering the decreased stage 2 sleep finding, it is reasonable to expect sleep spindles were likely decreased in the groups with Asperger's and ASD. These findings are

similar to the adult literature which indicates reduced spindles in populations with ASD (Godbout et al., 2000; Limoges et al., 2005).

Research by Godbout et al. (2000) showed adults with Asperger's had decreased sleep spindle density. Limoges et al (2005) examined sleep spindles in adults with Asperger's, ASD, and TD controls, in an overnight sleep study. Comparable to the findings with Godbout et al. (2000), the group with Asperger's and ASD had significantly reduced sleep spindle density and overall fewer sleep spindles. Godbout et al. (2000) exhibited reduced sleep spindles during Stage 2 sleep, suggesting reduced density in fast spindles, though this is speculative. Unfortunately, neither of these studies mentioned differences in fast or slow spindle frequencies, and thus it is unclear if there are differences in spindle frequencies in adults with ASD.

Despite the limited research examining sleep spindle characteristics in ASD research and the modest participant samples, findings are relatively consistent across age and research studies. These findings suggest features of sleep spindles are altered in adults with ASD and Asperger's (Godbout et al., 2000; Limoges et al.,2005), which is consistent with the present study showing decreased slow and fast spindles and increased middle spindles. A novel finding in this study was increased middle spindle frequencies in the group with ASD. Both primary and secondary analyses showed an increase in middle spindles which is likely due to decreased slow and fast frequencies and thus, the variance of spindle frequency is reflected in the middle spindle band. In other words, the group with ASD either had slower fast spindle frequencies, appearing more like a middle spindle or in some cases, individual participants with ASD did not have a spindle peak frequency. Overall, these findings suggest the neural networks are different in populations with ASD (Ghuman et al., 2016) and these differences are present during NREM sleep in infants and toddlers.

SWA. SWA was also examined for group and individual differences in ASD. Surprisingly, neither the primary or secondary analyses detected any differences in SWA. This may be due to the fact that across all participants, nap recordings were primarily composed of NREM sleep with similar amounts of Stage 2 (known for presence of fast spindles) and Stage 3 sleep (increased SWA and presence of slow spindles). Delta activity, an indicator of SWA, was similar across groups at both the group and individual level. This is the first study examining SWA in infant/toddlers with ASD. It is conceivable that overnight sleep recordings are better suited to capture possible differences in SWA, however, one overnight study in adults with ASD also showed no decrease in Stage 3 sleep, where SWA is most prevalent (Godbout et al., 2000).

One recent study has examined delta activity, a marker of SWA, in adults with ASD (Rochette, Soulieres, Berthiaume, & Godbout, 2018). Unfortunately, this study did not examine SWA, however, compared to the control group, adults with ASD had significantly less delta activity in parieto-occipital regions and decreased delta in frontal to posterior regions. This suggests that individuals with ASD may have decreased SWA. These findings contradict the findings from the present study and Godbout et al. (2000). While research in populations with ASD show decreased sleep spindles, SWA has yet to be fully examined in both adults and children with ASD and thus, more research with overnight recordings is needed to better understand the role of SWA across ASD in early childhood.

Other Frequencies During NREM and ASD. Two interesting and consistent findings across primary and secondary analyses differentiated infants and toddlers with ASD and those who were TD. At both the group and individual level, there was decreased power and density in fast theta (5.25-7 Hz) and increased beta (20-25 Hz) activity. Although no other studies investigating NREM sleep and ASD report any differences in theta or beta, these findings are

consistent with the literature examining wake resting state (RS) EEG in ASD (Bink, van Boxtel, Popma, Bongers, Denissen, & van Nieuwenhuizen, 2015; Frohlich et al. 2016; Kozhushko et al., 2018). Across school aged children to adulthood, excessive power in frontal and right posterior cites in theta (Pop-Jordanova, Zorcec, Demerdzieva, & Gucev, 2010) is widely reported. In the present study, infants and toddlers with ASD and at-risk for ASD showed decreased theta and in some instances, participants did not show a theta peak. Because the present study examined NREM sleep, this could explain the power differences in theta activity that are observed in the wake state. Another reason for this difference is this study examined infants/toddlers. Findings from research question 1a showed power in theta increased over the beginning years of life and thus, slower theta in ASD may suggest a delay in theta activity. Research examining NREM features in older populations, show theta is absent during NREM sleep (Campbell & Feinberg, 2003) and thus, theta activity could be specific to early childhood during NREM sleep. Given the findings of the current study and consideration for the body of research examining RS EEG, differences in theta activity seem to be a defining feature of ASD.

Similar to the findings in the present study, differences in higher frequencies as beta have been shown during RS in ASD (Bink, van Boxtel, Popma, Bongers, Denissen, & van Nieuwenhuizen, 2015; Frohlich et al. 2016; Kozhushko et al., 2018; Wang et al. 2016) and duplication of 15q11.2-q13.1 (Dup15q syndrome) a genetic variant of ASD (Frohlich et al. 2016). In the present study, increased beta was found in both spectral power and density, and this was present in both primary and secondary analyses at the group and individual level. Though less robust, Figures 6 and 7 revealed remarkably similar topographic patterns of beta activity across all skillsets. The primary analyses showed increased beta activity across all domains on the VABS and all domains on the MSEL, except Expressive and Receptive Language (MSEL)

showed trend level correlations. Secondary analyses revealed widespread correlations with beta and all domains on the MSEL and VABS. Again, this is the first study elucidating differences in beta activity during sleep and specifically, NREM sleep. Research with young children with genetic variants of ASD as Dup15q syndrome and idiopathic ASD has shown excessive beta activity during RS EEG (Frohlich et al, 2016; Kozhushko et al., 2018). Thus, increased beta activity may be a defining feature of ASD that is not solely restricted to one state as wake or sleep. Moreover, with a larger sample this may further distinguish if the increased beta activity is specific to NREM. The secondary analysis provides support for this last point, yet caution is warranted as some participant's data were used twice and thus this could inflate some of the findings with beta.

Across studies, EEG differences are found in various populations with ASD. These differences are seen in theta, sleep spindle, and beta bands. These features are present across development and populations with different variations of ASD. More research is needed to discern which frequencies are specific to idiopathic ASD and which frequencies may be specific to genetic variants as Dup15q syndrome. Furthermore, more research is needed to determine if these frequencies are specific to the sleep, wake or both conditions.

ASD Symptomatology. Findings showed participants with high scores on the ADOS-2 or rather increased symptom severity of ASD showed lower performance on the MSEL and VABS. To further examine symptom severity of ASD and features of NREM sleep, participant's (ASD and TD) performance on the ADOS-2 was correlated with the differentiating NREM features, of fast theta (5.25-7 Hz), middle (11-14 Hz) and fast (14.75-15.25 Hz) spindles, and beta (20-25 Hz). The primary analysis showed trend level negative correlations with theta and the ADOS-02 and a modest left positive correlation with middle spindles and the ADOS-2. The

secondary analysis showed slightly different findings where theta was negative correlated with a left temporo-occipital cite and middle spindles exhibited trend level positive correlations. Both primary and secondary analyses exhibited negative correlations with fast sleep spindles in frontal regions based on ADOS-2 scores. Participants with low scores on the ADOS-2 had faster (frequency) and more (density) sleep spindles. Widespread positive correlations with beta and the ADOS-2 were found and thus, participants with high scores on the ADOS-2 had significantly more beta than participants who scored low on the ADOS-2. Increased beta activity has been widely reported during wake RS in populations with ASD (Bink, van Boxtel, Popma, Bongers, Denissen, & van Nieuwenhuizen, 2015; Frohlich et al. 2016; Kozhushko et al., 2018). In the wake state, beta is thought to reflect active task engagement, alertness, and motor behavior (Wang et al., 2016). Though this is the first study examining associations with autism severity and features of NREM sleep, beta may be an interesting and understudied rhythm during NREM and an oscillation to pursue further.

NREM Associations with Development in ASD

Another unique feature of this study was examining NREM sleep and associations with development and risk in ASD. Only a handful of studies have examined NREM sleep in ASD and only a couple of studies have examined NREM associations with behavioral measures (Tessier et al., 2015). This is the first study to examine NREM sleep and behavioral performance with traditional measures of child development in infants/toddlers in ASD. Domain scores and overall standard score (composite) on the MSEL and the VABS were compared with NREM frequencies shown to differentiate ASD and TD, which were theta, sigma (sleep spindles), and beta.

Primary and secondary analyses confirmed that one main difference between groups was performance on the MSEL. This difference may be due to the infants and toddlers with ASD and the infants and toddlers at-risk of ASD likely having delays or other comorbidities that have not been identified. For example, both analyses suggest that for some participants with ASD, the low MSEL quotient could suggest possible comorbidity of an intellectual disability. It may be that some of the correlations are capturing characteristics of developmental delay/disability rather than merely ASD.

Performance on the MSEL and VABS were correlated with fast theta (5.25-7 Hz), middle spindles (11-14 Hz) and fast spindles (14.75-15.25 Hz), and beta (20-25 Hz). Both primary and secondary analyses revealed participants with higher performance on the MSEL had increased fast theta and fast spindle activity and reduced middle spindle and beta activity. Thus, the TD group showed faster theta and more fast and slow spindles. By contrast, participants with lower performance on the MSEL showed decreased theta, increased middle spindles, and increased beta activity.

Overall, findings on the VABS are similar to the findings on the MSEL. Primary and secondary analyses showed either trend level or statistically significant findings in participants with increased fast theta and fast sleep spindles, while slow spindles and beta were negatively correlated with performance on the VABS. In other words, participants with higher performance on the VABS showed faster theta and sleep spindles and reduced middle spindles and beta frequencies. Participants with lower scores on the VABS had both increased middle spindles and beta activity.

Of the two studies examining behavioral correlations with NREM sleep and ASD, these two studies solely examined sleep spindles and thus, research has largely ignored the possibility

of contributions from other frequency bandwidths. Limoges et al., (2013) examined adults with ASD during an overnight sleep recording and examined their performance on a measure of IQ and a sensory-motor procedural memory task. Sleep spindles were negatively associated with both the number of trials needed to learn a sensory-motor procedural memory task, and reaction time in the same task. In other words, fewer sleep spindles were associated with more trials to learn a task, increased reaction time to complete the task, and increased errors when performing the task. Tessier et al. (2015) also found similar findings with sleep spindles and school age children with ASD. Verbal IQ and full-scale IQ were negatively correlated with spindle density in the ASD group. Unfortunately, these studies did not separate the sigma band into slow, medium or fast spindle frequencies, thus it is unclear if slow or fast spindles or both are negatively correlated with performance. Primary and secondary analyses in the current study also found negative correlations with performance on the VABS and MSEL in specifically slow and fast spindles. Thus, the present study extends the research findings of Limoges et al., (2013) and Tessier et al., (2015) and provides further support for decreased sigma activity (sleep spindles) as a distinguishing feature of ASD.

Summary. The findings of the present study consistently show that participants with ASD have reduced theta and slow and fast spindles and increased middle spindles and beta activity and for the most part, these rhythms are negatively associated with performance on the MSEL and VABS. Despite the small age matched comparison, similar results were found in a slightly bigger sample. The majority of the primary and secondary analyses revealed remarkably similar significant differences between ASD and TD in theta, sleep spindles, and beta activity. There was only a slight variation in some of the findings between the primary and secondary analyses and these were found in the correlations with theta and Expressive and Receptive

Language and Visual Perception (MSEL). The primary and secondary analyses also showed a slight change in correlations with theta and ADOS-2 and middle spindles and ADOS-2 where one analysis revealed trend level differences and the other presented statistically significant differences.

Given the subtle variation in the findings from the primary and secondary analyses, both analyses suggest different NREM patterns in infants and toddlers with ASD.

Since this is one of the first studies characterizing these findings, it is difficult to interrupt the precise nature of these results. Specific frequency bands are thought to govern specific mental states. For example, theta is associated with attentional states, and sigma band (sleep spindles) has been associated with learning, motor, and declarative memory (De Gennaro & Ferrara, 2003; Luthi et al., 2014), while beta is correlated with the processing of motor behaviors (Ewen et al. 2016). Thus, these findings suggest that the observed NREM differences and associations with performance on measures as the MSEL and VABS in ASD, are due to alterations in power (microvolt^2) and density (number/min) in these specific frequencies. These specific alterations have been shown to be relatively stable across age and populations with ASD and may not solely be specific to NREM sleep in ASD. Given the limited research on NREM sleep and ASD, more research is needed to understand the role of NREM sleep in development and cognitive performance in ASD and moreover, the role of NREM as a potential biomarker in ASD.

NREM Sleep: A Potential Risk Marker for ASD

The establishment of a biomarker(s) used to differentiate infants at-risk of ASD, before the onset of symptoms, may be a fruitful strategy for research directed at understanding the neurological underpinnings of ASD (Jeste, Frohlich, & Loo, 2015; Varcin & Nelson, 2016). A biomarker suggests risk well before behavioral symptoms are apparent and thus, may serve as a

screening marker used to predict risk status (Varcin & Nelson, 2016). Biomarkers are put forth at the molecular level, gene expression, eye movement, head size, and brain function (Walsh et al., 2011. The most widely studied markers in infants/toddlers are brain function. These markers are typically used with measures such as EEG, magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI). Due to clinical heterogeneity (Jeste, Frohlich, & Loo, 2015; Walsh et al., 2011), poor participant descriptions (Wang et al., 2013), and methodological inconsistencies (Wang et al., 2013), research has yet to establish a biomarker of ASD.

Research using EEG as the primary measure can be difficult for determining a biomarker. Despite research widely using EEG or hdEEG in infants/toddlers at-risk of ASD for detecting biomarker(s), previous studies have used the wake state to examine an event related potential (ERP) or a resting state (RS) EEG (Gabard-Durnam et al., 2015; Luyster et al., 2011; Tierney et al., 2012; Varnin & Nelson, 2016; Webb et al., 2010; Webb et al., 2011; Webb et al., 2012; Webb et al., 2015). There are some concerns with these methods, and the most obvious being infants/toddlers don't sit still for a task, and thus artifacts and noise are created. A second concern, is that one may not "truly know" if the infant is attending to the presented stimuli or alternatively, the infant is attending to some extraneous factor when they should be at "rest". Thus, sleep was put forth to address some of these concerns as a potential risk marker of ASD. The current study assessed the extent to which differences were found in the 12-30-month age range that may be used to detect risk of ASD well before symptoms become apparent at the behavioral level. This is the first study examining sleep spindles, SWA, and other frequencies present during NREM sleep and risk for ASD. Several findings differentiated ASD from TD. These main findings were differences in theta, sleep spindles and beta activity which were negatively associated with cognitive performance on the MSEL and VABS. Despite the small

sample, these findings were consistent and thus, have multiple implications for biomarkers with EEG in research and clinical practice.

Considerations for Research. In many cases, it is presumed that a biomarker must associate risk, predict diagnosis, and have observable change after treatment (Jest, Frohlich, & Loo, 2011). As research acknowledges the early plasticity of the brain with sensitive and critical periods of development (Varcin & Nelson, 2016), it is a huge endeavor for a biomarker(s) or a subset of markers to associate risk, predict diagnosis, and exhibit change from treatment. Given the dimensions of a biomarker, it seems important to first establish biomarkers within each subset (levels) and then assess if these markers are complex and multidimensional to predict across levels (risk, diagnosis, treatment) and if these markers are stable over time. In the current study, there were clear differences shown to differentiate TD and ASD. However, this study employed a cross sectional design and thus did not capture the development of NREM sleep at multiple timepoints. This is an important concern that should be addressed in future research.

Other considerations to examine are the malleability of biomarkers and understanding trait (features specific to the individual) or state (features that change such as tasks, mood, medications, etc.), and if these features are stable or change over time. Research with EEG and ERP show differences in connectivity at various ages in development (Wang et al. 2013) however, it is unclear which changes are sleep or wake specific, what are maturational specific, and what alterations are specific to ASD. Beta activity is a prime example of this last point. Excessive beta is widely examined in the wake EEG and until now, beta has yet to be studied in NREM sleep. Thus, it may be the case that the increased beta activity may not be specific to wake or sleep but may be present in both.

A final consideration for research directed at establishing a biomarker of ASD, is that ASD has been around likely before Kanner's initial description (Wing, 1997). The previous Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000) included 'autistic disorder', 'Asperger's disorder' and 'pervasive developmental disorder not otherwise specified' (PDD-NOS) as categories of autism. Today, the DSM-V uses the umbrella term of autism spectrum disorder (American Psychiatric Association, 2013). Thus, as research seeks to capture what is now called ASD, there is greater emphasis on defining and demarcating subgroups of ASD. Moreover, not only is ASD characterized as a heterogenous neurodevelopmental disorder, individuals with ASD often experience comorbidities with other disabilities or disorders later on in life (Walsh et al., 2011; Wang et al., 2016). In the current study, the individuals in the ASD group may likely have a comorbidity that has yet to recognized. Unfortunately, due to the small comparative sample in both primary and secondary analyses, it is unclear if some of the observed differences are specific to ASD or if the findings are capturing what may be variations in development or other developmental disabilities. Thus, future research is needed to better distinguish what is specific to ASD and how these specific traits may inform the way in which ASD is captured.

Considerations for Clinical Practice. The establishment of a biomarker with EEG to differentiate infants at-risk of ASD before the presence of behavioral symptoms has great potential for clinical research and practice. Although research acknowledges this potential, there seems to be a gap in our practice and understanding of the feasibility of a marker that can be used in a clinical setting. For example, in the case of EEG, teaching a clinician to apply a hdEEG net is relatively easy, yet preprocessing, sleep scoring, and analysis of the EEG takes expertise and considerable amount of time. Moreover, EEG is one of many methods being used to detect a

biomarker. Thus, as research seeks to establish risk marker(s) of ASD, increased attention to the application and measurement in clinical settings is needed.

Research has yet to address questions of the applicable nature of a biomarker in clinical and applied settings and the extent to which a biomarker may afford access to clinical and intervention-based services (Dawson et al., 2012). Under the Individuals with Disabilities Education Act (IDEA) (IDEA, 2004), Part C services, a child under the age of 3, showing significant symptoms and/or a significant delay in a domain (such as language), is eligible for early intervention (EI). In all states, certain EI services are free (or filed through insurance) and if families want to participate, they cannot be denied services if they cannot afford to pay for them. Thus, with a risk marker that consistently detects neural differences occurring during or before the presence of behavioral symptoms, such as excessive beta activity and reduced sleep spindles, this could suggest risk for ASD and eligibility for intervention services. With a risk marker in place, clinicians and researchers could further examine the benefits for a child and their family of receiving services at an earlier age when the brain is most plastic (Varcin & Nelson, 2006). A risk marker would further allow research to examine the benefits of EI at both the neurological and behavioral level. This would provide insight into which interventions are effective and moreover, which populations benefit the most.

ASD and other neurodevelopmental disorders are often examined as something that needs to be fixed or attenuated and this may be hurtful to individuals, families and friends of people with ASD who may not view their development or characteristics as a disability. As research seeks to uncover the neurological underpinnings of ASD, more emphasis on the application and discussion of a biomarker are needed. In clinical settings it is important to talk with individuals with ASD, families, friends, and community stakeholders that work with

families and insurance companies to better understand how a marker can be used as an indicator of risk rather than a label. Having open conversations with caregivers about a biomarker and determining if and how a marker of ASD may or may not impact development and quality of life are important. Some families may be opposed to having their child examined for a marker. Whereas other families, particularly those who have an older child with ASD, may be interested in knowing their young child's risk for an eventual diagnosis of ASD. It may be the case that ASD is an example of neurodiversity (Glannon, 2007) and that variations in neurological development are a product of evolution. Whether this is the case or not, this should not undermine the values, experiences, and identities of individuals and their families with ASD. Thus, the next and most important step is to create platforms for open communication about risk markers. Only then, can research and clinicians truly begin to address questions of what constitutes a biomarker and how this can be applied.

Research Limitations

Though the results of this study provide a novel perspective related to early development and sleep, more research is needed to better understand the role of NREM sleep and early development at both the neural and behavioral levels. The present study used a cross sectional design providing a snapshot of development. Whereas a longitudinal study would be best suited to capture the significant intraindividual variance and immense change over time. Previously, one small longitudinal nap study (Kurth et al., 2016) was conducted with 8 children at 2, 3, and 5 years and noticed the sleep architecture was stable across age with similar patterns of prominent slow wave activity (SWA, 0.75-4.5 Hz), high theta (4.75–7.75 Hz) and sigma (10–15 Hz). Given the many differences found in the present study, it is important that longitudinal studies assess development in frequent shorter bouts to capture the subtle nuances that were likely missed by

this large time span and small sample size. This is particularly important when capturing the early onset of ASD as the presence or consistent absence of certain behaviors (Baranek et al., 1999) is important when identifying ASD.

A second limitation of the present study was that it solely examined NREM sleep and thus REM sleep was not examined. One constraint was that EMG was not recorded and only a few participants had high-quality EOG data. Without EMG and EOG, it is difficult to identify REM with certainty. A third constraint of this study was that all recordings occurred during a midday nap, and thus most infants had only NREM sleep. To better capture the circadian influence, overnight recordings are needed to better describe the transitions between NREM and REM. Given that this study involved recruitment of young children and it was important to acknowledge both the family and child's schedule and routines, families were asked to arrive during their child's typical naptime. As such, it is necessary to consider circadian influence of different nap times. However, after controlling for time of the nap, age related changes remained but nonetheless this is an important consideration.

Another limitation and consideration for future studies is gender differences. Given that ASD is more prevalent in males, it is not surprising that the group with ASD had more males than females. Despite the difference in gender, gender was not statistically different in this study.

A final limitation was this study had a modest participant sample and a particularly small sample of infants/toddlers in the ASD group. Although the age matched sample of 7 and 7 did yield statistically significant differences between ASD and TD, the resampling method used for the secondary analyses, provides further support for the benefits of an increased sample size which allowed for increased statistical power. Although there are some concerns with this re-sampling technique as non-independent samples (Vaden, Gebregziabher, Kuchinsky, & Eckert,

2012) this does suggest that more participants could allow for additional distinction between groups. Moreover, with the ASD group, all of the children met criteria for ASD on the ADOS-2. Despite meeting criteria, half of the children had a formal diagnosis whereas the other half were genetically at-risk and also met criteria for ASD. Furthermore, one of the participants in the ASD group was not born full term. Research has widely shown that risk factors as preterm birth (⩽34 weeks of gestation, Guinchat, et al. 2012; Larsson et a., 2005; Trevaud et al. 2013), low birth weight (Gray, O'Callaghan, & Gibbons, 2015), and other such factors as increased head circumference (brain volume and weight; Redcay & Courchesne, 2005) are associated with a variety of developmental disorders, including ASD. Thus, a larger sample is needed for targeting subgroups allowing for increased precision and distinct populations (Chawarska et al., 2014; Zwaigenabum et al. 2016), rather than capturing what may otherwise be developmental delay/disability.

Future Directions

In the future, research examining NREM sleep should incorporate longitudinal methodology to capture early development and sleep. Longitudinal studies are best suited to capture the significant inter-and-intraindividual variance and immense change that is present in early development (King et al., 2017). Due to many logistical constraints such as time and money, the present study employed a cross sectional design, and thus, only captured a glimpse of development. Longitudinal designs are particularly important when capturing the early onset of neurodevelopmental disorders, such as ASD. Symptoms of ASD may not be present at specific ages or children may show signs of regressed development or plateaued. Thus, future studies would benefit from multiple measurements across developmental periods (Kurth et al., 2010).

Future research would also benefit from overnight sleep recordings. Overnight recordings allow for the following: (1) observed fluctuations of REM and NREM sleep, (2) progression of SWA, (3) influence of the circadian cycle, and (4) a more refined picture of the full sleep cycle. Given logistical restraints and respecting the time and needs of the family, the present study used day time naps. Considerable time and attention is needed to work with families and their schedules. This research could be conducted in families' homes and this would be more representative of an early intervention model (Part C services) in which families receive services within their natural environment.

Future research is needed to compare development across various categories of developmental delay/disability. Such a comparison could increase the sample size of the study and thus increase statistical power. The present study had a modest sample of TD infants/toddlers and a smaller group of infants/toddlers with or at-risk for ASD. The resampling of participants was used to explore the benefits of a larger sample size. One concern with this method is non-independent samples. Thus, it is unclear if some of the most robust findings are due to the non-independence of the data or increased sample size. Furthermore, including children with identified developmental delays/disabilities would allow for further demarcation of subgroups (Walsh et al. 2011). This would provide credence for the interesting findings within the present study and pinpoint whether the present findings are more reflective of ASD or overall developmental delay. This would also provide support for research examining potential biomarkers of ASD (Walsh et al., 2011; Varcin & Nelson, 2006). More research with infants and toddlers with various risk factors and predispositions for developmental delay would provide valuable insight into the underlying etiologies of neurodevelopmental disorders.

Although this was not an aim of the present study, future research is needed to incorporate more measures about the family. Although the present study used a Brief Information Questionnaire (BIQ) to examine family demographics, this did not capture the influences and the important role of the family. Sleep routines are often set by the family and the family's schedule (Mallow et al., 2009). Thus, the physiological features of sleep can be altered by the family's day-to-day activities and routines. This has important implications for intervention research, and clinicians working with families and young children to incorporate sleep routines, and thus it is important to see what factors are contributing to the child's sleep and overall quality of sleep (Mallow et al., 2009).

Finally, after a search of the literature using the following keywords: autism or ASD, biomarkers or endophenotypes, family perspectives or parent perspectives, family perceptions or parent perceptions, and clinical practices, the results demonstrated a paucity of research. The lack of research on linking families' perceptions around biomarkers suggests that research is primarily focused on one aspect and this is to detect a biomarker, and thus, research has not given ample consideration for familial factors and the application of a marker. Thus, it is unclear how families, communities, and society will handle the adoption of a marker and thus, this needs to be addressed. Qualitative research using focus groups with physicians (Crais et al., 2014), families, and community stakeholders to examine perceptions of clinical biomarkers for determining risk and obtaining services would provide valuable and needed insight.

Conclusion

This study contributes to our understanding of the sleep topography in the transition from infancy to toddlerhood. This is one of the first studies to characterize development through the lens of NREM sleep with behavioral and physiological data in the 12-30-month age span. This is

also the first study using behavioral and NREM physiological data from infants/toddlers to examine risk of ASD. Elucidating the role of NREM sleep is an important step for the identification of a potential risk marker of ASD. The findings reported here are applicable to research seeking to understand development in this age range and the neurological underpinnings of ASD.

The most important findings from this study are the NREM characteristics reflected in the sleep EEG showing changes with age. Many of these NREM frequencies were highly correlated with a number of domains on the MSEL and VABS as Fine Motor, Language, and Socialization in the 12-30-month age range. Moreover, primary and secondary analyses examining features of NREM sleep showed significant differences between infants and toddlers with or at-risk of ASD and TD. These differences were decreased power and density in fast theta and sleep spindles, and increased power and density in the middle spindle and beta frequencies in ASD. The differences in theta, sleep spindles, and beta band EEG were highly correlated with performance on the MSEL and VABS.

Despite the many noted differences that distinguished children with ASD from TD, the need for a biomarker may not be as important as the need for the conversation about the implications of a biomarker for research and clinical practice. More research is needed to better understand the role of families, communities, and policymakers in the identification of a biomarker and most importantly what it means for individuals who may be characterized as at-risk by variations of a biological or physiological feature. These are important ethical and practical considerations that have yet to be examined.

APPENDIX A: DEFINITION OF KEY TERMINOLOGY

10-20 system- Universal head measurement and application of electrodes. Placement of electrode site has a letter to denote area of the lobe and includes Frontal (F), Temporal (T), Parietal (P), Occipital (O), and Central (C). Placement is also described in relation to the midline saggital plane and include Fz, Cz, Oz, where "Z" means zero. (Iber et al., 2007)

Alpha (α)- Bandwidth between 8-14 Hz. Often observed during wake sometimes characterized in sleep. (Frohlich, 2016)

Autism spectrum disorder (ASD)- A neurodevelopmental disorder characterized by impairments in social communication and restricted/repetitive behaviors (DSM-5; American Psychiatric Association, 2013)

Beta (β)- Bandwidth between 15-30 Hz involved with motor activities and alertness (Başar, Başar-Eroglu, Karakaş, & Schürmann, 2001).

Delta (δ)- A marker of slow wave activity characterized by slow oscillations (0.5-4 Hz) and increased amplitude. (Iber et al., 2007)

Experience-dependent plasticity – The ability of the brain to adapt to a changing environment (Wilhelm et al., 2014)

Fast Fourier Transformation (FFT)- The Fourier are techniques used convert a signal from its original domain such as time or space to the frequency domain (Hz) (Banaschewski, & Brandeis, 2007; Frohlich, 2016)

Gamma (γ)- Bandwidth > 30 Hz and observed during wake and important in early sensory experience and binding of information (Rojas & Wilson, 2014; Strzelecka, 2014)

Infant- Chronologically, an infant is 0-12 months. Understanding that development varies, here, infancy includes 0-15 months.

Sigma (Σ)– Bandwidth (10-16Hz) observed during NREM sleep and hallmarked by the presence of sleep spindles.

Sleep Spindles- Waxing and waning of the EEG between 10-16 Hz occurring in frontal or central areas. Can be fast or slow and known to change with age (De Gennaro & Ferrara, 2003).

Spectral density- Decomposing a complex signal into simpler parts (Achermann, 2009).

State- Variation or susceptible to alteration under circumstance or situation-specific (Chen et al. 2000).

Synchronized neural activity- Signals oscillating at the same frequency within regions (Nunez & Srinivasan, 2006).

Theta (θ)- Bandwidth between 4-8 Hz occurring during REM or NREM sleep and states of relaxation (Iber et al., 2007).

Toddler- Chronologically a toddler is 12-36 months. Understanding that development varies, here, toddler includes 15-36 months.

Topography- The mapping of electrical surface activity across the brain

Trait- Relatively reproducible feature across time and that is not affected by certain situations or conditions (Chen et al., 2000).

APPENDIX B: RECRUITMENT FLYER

Early Development and Sleep

Does Your Baby Take a Nap?

- **Who:** Infants/toddlers between 12 – 30 months of age
- **What:** Free developmental activities and Non-Invasive brain recording during your child's nap
- **When:** Activities and nap scheduled at your convenience
- **Where:** UNC Medical Wings Building C, room 242

Receive up to $75 for participating

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

If interested, please email or call us at:

EDS@med.unc.edu
919-445-0227

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APPENDIX C: RECRUITMENT FLYER

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

FROHLICH LAB

Research Study Opportunity

Early Development & Sleep Study

In this study we are examining sleep characteristics in infants/toddlers who are typically developing, delays in language, social communication and play, have autism or who are at-risk for autism (have a sibling with autism).

Why are we studying this?

Children develop and change rapidly during infancy and toddlerhood. We know that sleep is a critical aspect of health and brain development. However, little has been done to document patterns of sleep in young children or examine how brain activity during sleep may differ in children a delay in language, communication, play, or with autism.

You may be eligible if your family has an infant/toddler 12 –30 months with:

- Language delays (i.e., no words at 18 months, limited vocalizations at 12 months)
- Delays in social communication or play
- With an autism spectrum disorder.
- Who has an older sibling with an autism spectrum disorder.

What's involved?

- We schedule the two visits at your convenience. You will be with your child at all times.
- Home visit: The first visit is for a developmental assessment which involves your child playing with a variety of toys. You will be providing input about your child's development and sleep. This visit takes about 2 hours.
- Lab visit: You and your child will come to our nap room on campus. Your child will wear an EEG cap and take a nap. This is painless and non-invasive. This takes about 2 hours.

We are excited to hear from you!

Please give Jessica a call: (919) 445 - 0227

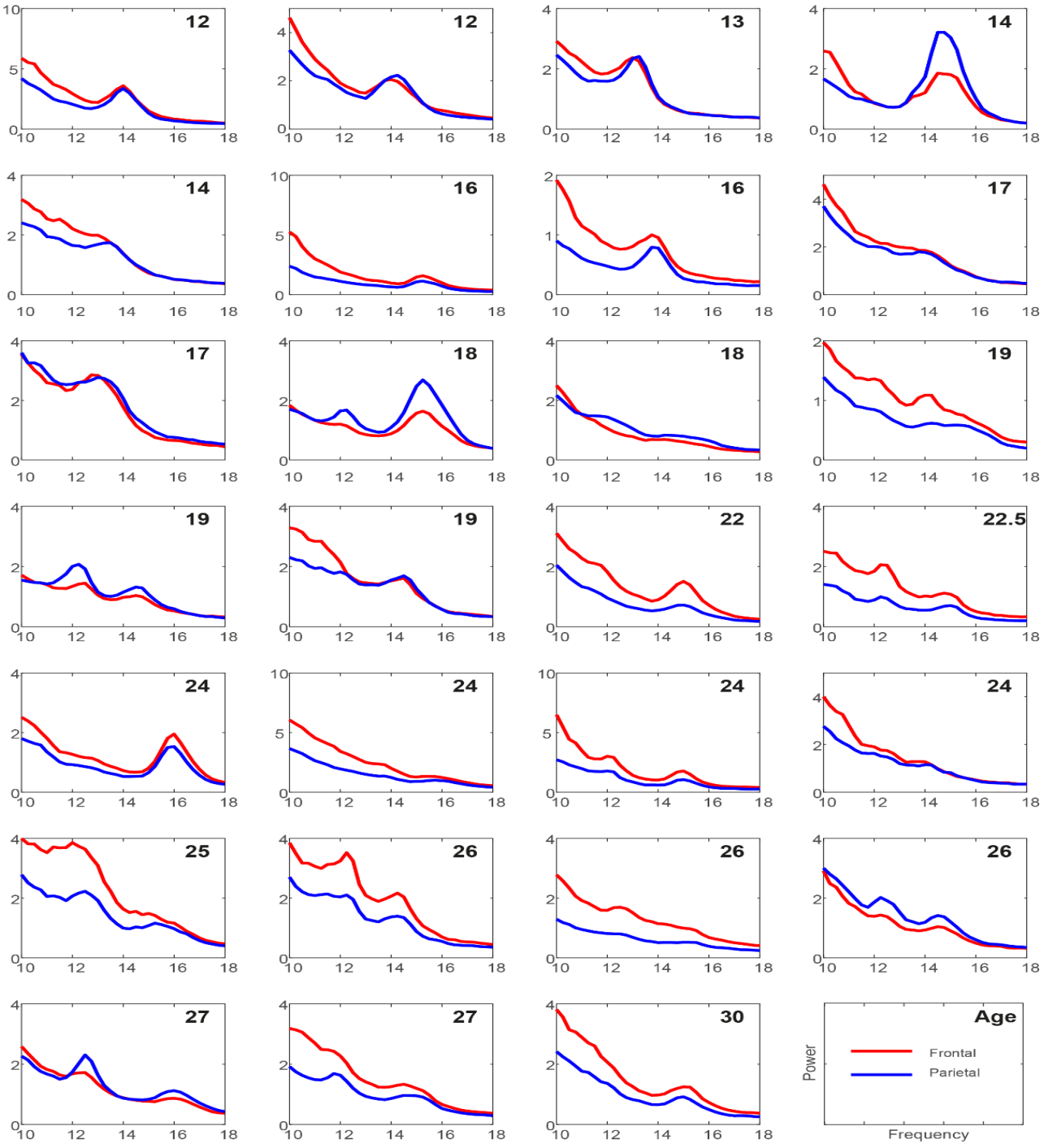
or email us at EDS@med.unc.edu to schedule your visit.

We are located at :

UNC Medical Wings with free parking

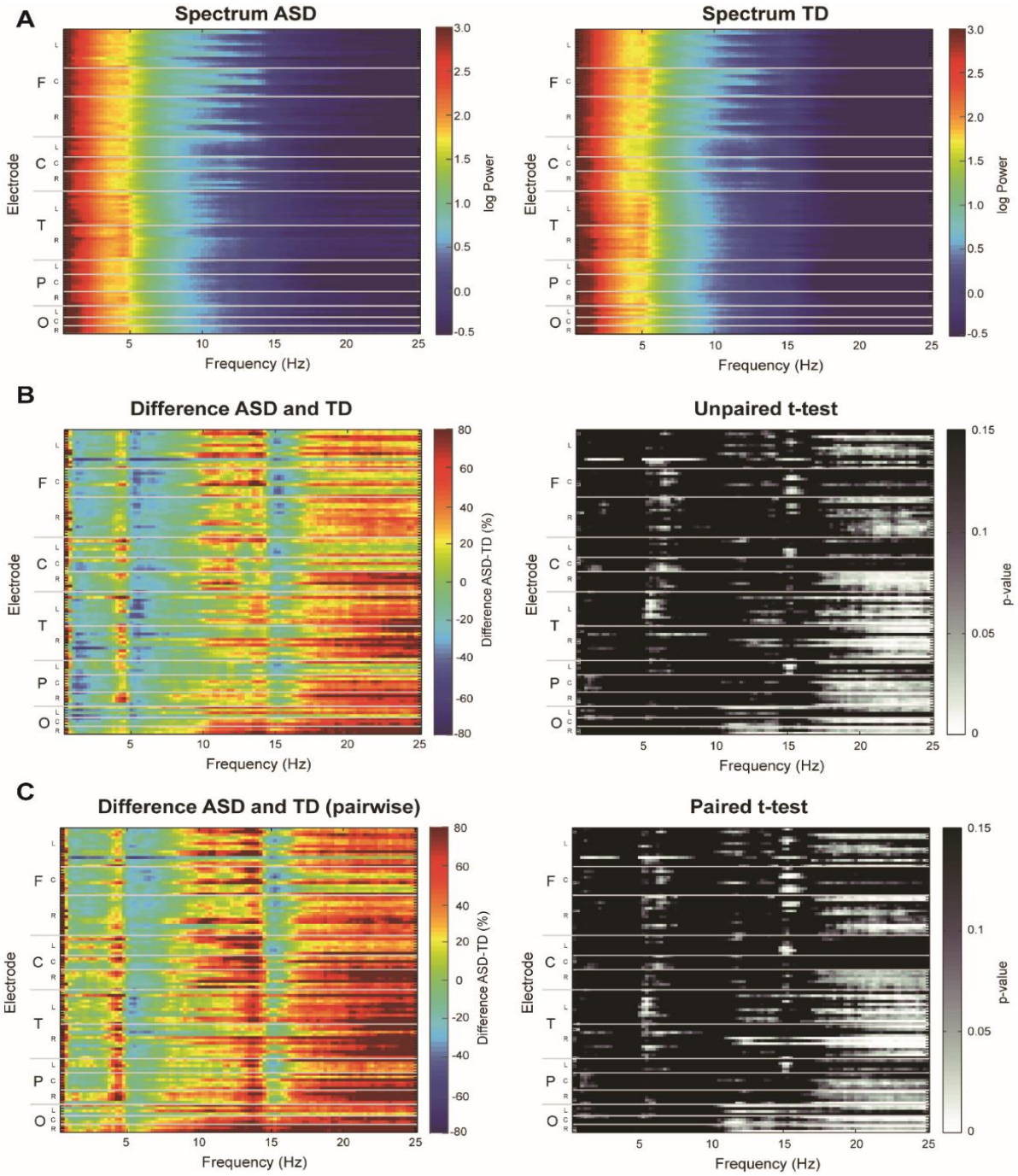
UNC Psychiatry Department

APPENDIX D: INDIVIDUAL SPECTRAL DENSITY PLOTS



Individual spectral density plots for all typically developing participants arranged youngest to oldest. Red: Frontal regions. Blue: Parietal regions.

APPENDIX E: RESAMPLED SPECTRAL POWER



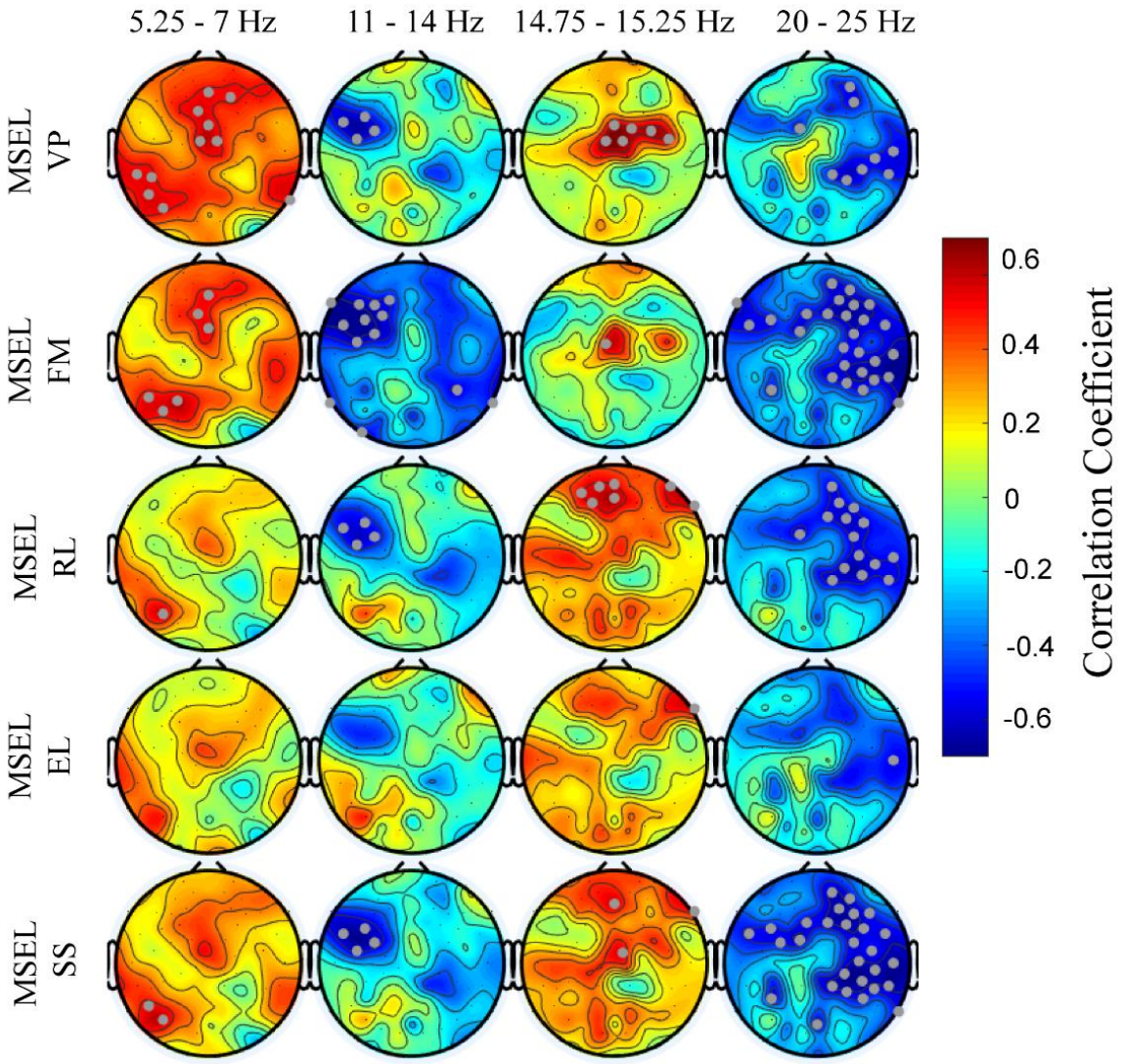
(A) Spectral power across all participants for ASD (first row, left) and typically developing (TD) comparison (first row, right) (B) Heat map of the difference of NREM spectral power values during NREM in ASD and TD. Left: Difference in spectra power are depicted with warm and cold colors, respectively. Second Row Right: unpaired t-test. (C) Heat map of the difference of NREM spectral power values during NREM in ASD and TD (pairwise). Left: Difference in spectra power are depicted with warm and cold colors, respectively. Third Row Right: Paired t-test.

APPENDIX F: RESAMPLED PARTICIPANT PERFORMANCE

	TD group (n=10)		ASD group (n=10)		
	Mean	(SD)	Mean	(SD)	p-value
MSEL	100.10	13.33	73.90	16.70	0.001**
MSEL VP	47.80	6.60	39.20	6.97	0.021*
MSEL FM	52.30	10.33	39.00	14.75	0.068ǂ
MSEL RL	46.00	10.87	32.40	11.17	0.007*
MSEL EL	51.80	12.34	32.50	12.22	0.022*
VABS	91.20	11.62	80.40	12.12	0.121
VABS Communication	95.40	13.38	81.20	14.73	0.053ǂ
VABS Daily Living	90.10	13.03	70.60	19.44	0.031*
VABS Socialization	92.90	7.88	82.10	13.16	0.025*
VABS Motor	93.40	10.34	91.20	10.26	0.676

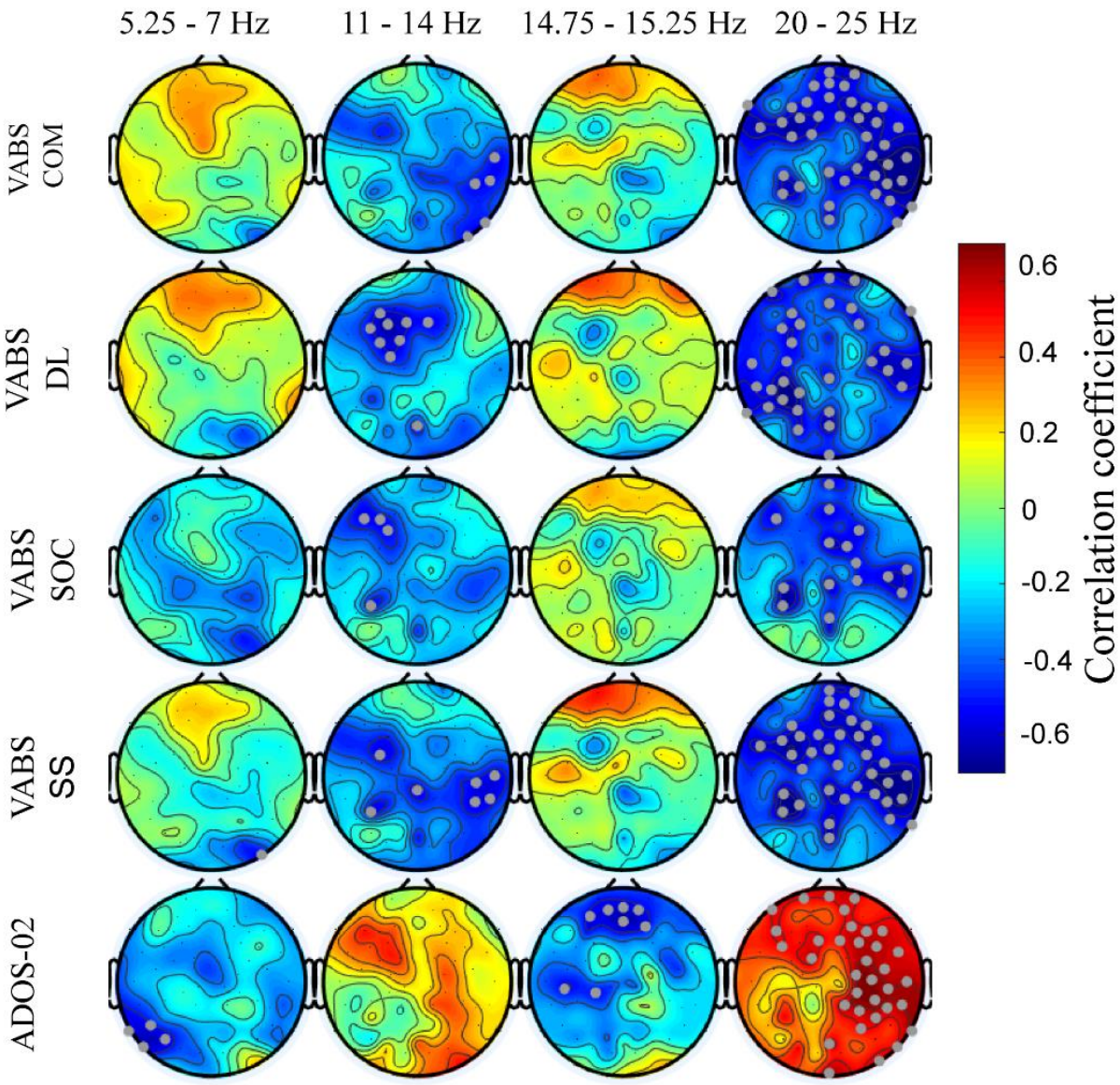
Trend level P value < 0.10ǂ, significant p value <0.05*, p value < 0.005**
MSEL-Mullen Scales of Early Learning Composite
VP=Visual Perception
FM=Fine Motor
RL=Receptive Language
EL=Expressive Language
VABS-Vineland Adaptive Behavior Scales- Second Edition composite
VABS Communication Domain Standard Score
VABS Daily Living Domain Standard Score
VABS Socialization Domain Standard Score
VABS Motor Skills Domain Standard Score

APPENDIX G: RESAMPLED TOPOGRAPHIC CORRELATIONS MSEL



Topographical representation of Pearson correlation coefficients between different bands of spectral power, MSEL measures and domains. Mullen Scales of Early Learning with subdomains (MSEL: Visual Perception, Fine Motor, Receptive Language, and Expressive Language, and the MSEL overall standard score). Electrodes that showed significant correlations after permutation statistical correction (SnPM) marked with grey dots.

APPENDIX H: RESAMPLED TOPOGRAPHIC CORRELATIONS VABS AND ADOS



Topographical representation of Pearson correlation coefficients between different bands of spectral power, cognitive measures and domains (corrected for age). Electrodes that showed significant correlations after permutation statistical correction (SnPM) marked with grey dots. Vineland Adaptive Behavior Scale standard score (VABS: Communication, Daily Living Skills, Socialization, and VABS Standard Score) and the Autism Diagnostic Observation Schedule (ADOS-02).

APPENDIX I: RESAMPLED PARTICIPANT CORRELATIONS WITH ADOS-2

Correlation	Measure	R	P
ADOS-2	MSEL Composite	-0.77	0.001*
	MSEL VP	-0.62	0.004*
	MSEL FM	-0.56	0.010*
	MSEL EL	-0.71	0.001*
	MSEL RL	-0.72	0.001*
	VABS Composite	-0.54	0.015*
	VABS Communication	-0.56	0.010*
	VABS Daily Living	-0.49	0.028*
	VABS Socialization	-0.59	0.006*
	VABS Motor Domain	-0.30	0.015*

Trend level P value < 0.10ǂ, significant p value <0.05*
MSEL-Mullen Scales of Early Learning Composite
VP=Visual Perception
FM=Fine Motor
EL=Expressive Language
RL=Receptive Language
VABS =Vineland Adaptive Behavior Scales- Second Edition
VABS Communication= Communication domain standard score
VABS Daily Living= Daily Living domain standard score
VABS Social Domain= Social domain standard score
VABS Motor Domain= Motor domain standard score

REFERENCES

Achermann, P. (2009). EEG analysis applied to sleep. *Epileptologie, 26,* 28-33.

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *Journal of cognitive neuroscience, 13*(2), 232-240.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed.). doi:10.1176/appi.books.9780890423349.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC. http://dx.doi.org/10.1176/appi.books.9780890425596

Andrillon, T., Nir, Y., Staba, R. J., Ferrarelli, F., Cirelli, C., Tononi, G., & Fried, I. (2011). Sleep spindles in humans: insights from intracranial EEG and unit recordings. *Journal of Neuroscience, 31*(49), 17821-17834.

Arns, M., & Kenemans, J. L. (2014). Neurofeedback in ADHD and insomnia: vigilance stabilization through sleep spindles and circadian networks. *Neuroscience & Biobehavioral Reviews, 44*, 183-194.

Astill, R. G., Piantoni, G., Raymann, R. J., Vis, J. C., Coppens, J. E., Walker, M. P., . . . Van Someren, E. J. (2014a). Sleep spindle and slow wave frequency reflect motor skill performance in primary school-age children. *Frontiers in human neuroscience, 8*.

Astill, R. G., Piantoni, G., Raymann, R. J., Vis, J. C., Coppens, J. E., Walker, M. P., . . . Van Someren, E. J. (2014b). Sleep spindle and slow wave frequency reflect motor skill performance in primary school-age children. *Frontiers in human neuroscience, 8,* 910.

Baio, J., Wiggins, L., & Christensen, D. Autism and Developmental Disabilities Monitoring (ADDM) April 26th, 2018. 2018 Community Report from the Autism and Developmental Disabilities Monitoring Network. Retrieved from https://www.cdc.gov/ncbddd/autism/materials/addm-factsheet.html

Banaschewski, T., & Brandeis, D. (2007). Annotation: what electrical brain activity tells us about brain function that other techniques cannot tell us–a child psychiatric perspective. *Journal of Child Psychology and Psychiatry, 48*(5), 415-435.

Bang, J., Khalilzadeh,O., Hamalainen ,M, Watanabe, T., & Sasaki, Y.(2014) Location specific sleep spindle activity in the early visual areas and perceptual learning. *Vision Research*, 99,162–171.

Baranek, G.T. (1999). Autism during infancy: a retrospective video analysis of sensory motor and social behaviors at 9–12 months of age. *Journal of Autism and Developmental Disorder, 29*, 213–224.

Barbaro, J., & Dissanayake, C. (2009). Autism spectrum disorders in infancy and toddlerhood: a review of the evidence on early signs, early identification tools, and early diagnosis. *Journal of Developmental & Behavioral Pediatrics, 30*(5), 447-459.

Barton, M. L., Dumont-Mathieu, T., & Fein, D. (2012). Screening young children for autism spectrum disorders in primary practice. *J Autism Dev Disord, 42*(6), 1165-1174. doi:10.1007/s10803-011-1343-5.

Başar, E., Başar-Eroglu, C., Karakaş, S., & Schürmann, M. (2001). Gamma, alpha, delta, and theta oscillations govern cognitive processes. *International Journal of Psychophysiology, 39*(2), 241-248.

Bathory, E. & Tomopoulos, S. (2017). Sleep regulation, physiology and development, sleep duration and patterns, and sleep hygiene in infants, toddlers, and preschool-age children. Current Probl Pediatric Adolescence Health Care. (47), 2, 29-42. doi: 10.1016/j.cppeds.2016.12.001.

Bigdely-Shamlo, N., Mullen, T., Kothe, C., Su, K.-M., & Robbins, K. A. (2015). The PREP pipeline: standardized preprocessing for large-scale EEG analysis. *Frontiers in neuroinformatics, 9*, 16.

Bink, M.,van Nieuwenhuizen C, Popma A, Bongers IL, & van Boxtel G.J. (2014) Neurocognitive effects of neurofeedback in adolescents with ADHD: A randomized controlled trial.*Clinical Psychiatry.* 75(5):535-42. doi: 10.4088/JCP.13m08590.

Blake, H., & Gerard, R. W. (1937). Brain potentials during sleep. *American Journal of Physiology--Legacy Content, 119*(4), 692-703.

Bódizs, R., Gombos, F., Ujma, P. P., & Kovács, I. (2014). Sleep spindling and fluid intelligence across adolescent development: sex matters. *Frontiers in human neuroscience, 8*.

Boostani, R., Karimzadeh, F., & Nami, M. (2017). A comparative review on sleep stage classification methods in patients and healthy individuals. *Computer Methods and Programs in Biomedicine, 140*, 77-91.

Borb, A. A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *Journal of biological rhythms*, *14*(6), 559-570.

Borbély, A. A. (1982). A two process model of sleep regulation. *Human neurobiology*.

Bosl, W., Tierney, A., Tager-Flusberg, H., & Nelson, C. (2011). EEG complexity as a biomarker for autism spectrum disorder risk. *BMC medicine, 9*(1), 18.

Bruni, O., Ferri, R., Vittori, E., Novelli, L., Vignati, M., Porfirio, M. C., . . . Curatolo, P. (2007). Sleep architecture and NREM alterations in children and adolescents with Asperger syndrome. *Sleep, 30*(11), 1577-1585.

Buckley, Sassower, K., Rodriguez, A. J., Jennison, K., Wingert, K., Buckley, J., . . . Swedo, S. (2011). An open label trial of donepezil for enhancement of rapid eye movement sleep in young children with autism spectrum disorders. *Journal of child and adolescent psychopharmacology, 21*(4), 353-357.

Buckley, Scott, R., Tyler, A., Mahoney, J. M., Thurm, A., Farmer, C., . . . Holmes, G. L. (2015). State-Dependent Differences in Functional Connectivity in Young Children With Autism Spectrum Disorder. *EBioMedicine, 2*(12), 1905-1915.

Campbell, I. & Feinberg,I. (2009). Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. Proceedings of the National Academy of Sciences of the United States of America, 106(13), 5177–5180.

Chatburn, A., Coussens, S., Lushington, K., Kennedy, D., Baumert, M., & Kohler, M. (2013). Sleep spindle activity and cognitive performance in healthy children. *Sleep, 36*(2), 237-243.

Chawarska, K., Frederick S., Macari, S., Campbell, D.J., Brian, J., Landa, R., Hutman, T., Nelson, C.A., Ozonoff, S., Tager-Flusberg, H., Young, G.S., Zwaigenbaum, L., Cohen,

I.C., Charman, T., Messinger, D.S., Klin,A., Johnson, S., & Bryson, S. (2014). 18-Month predictors of later outcomes in younger siblings of children with autism spectrum disorder: a baby siblings research consortium study. J*ournal of the American Academy of Child & Adolescent Psychiatry, 53,* (12),1317-1327

Chen, G., Gully, S. M., Whiteman, J.-A., & Kilcullen, R. N. (2000). Examination of relationships among trait-like individual differences, state-like individual differences, and learning performance. *Journal of Applied Psychology, 85*(6), 835.

Chu, C. J., Leahy, J., Pathmanathan, J., Kramer, M. A., & Cash, S. S. (2014). The maturation of cortical sleep rhythms and networks over early development. *Clin Neurophysiol, 125*(7), 1360-1370. doi:10.1016/j.clinph.2013.11.028

Cirelli, C., & Tononi, G. (2004). Locus ceruleus control of state-dependent gene expression. *Journal of Neuroscience, 24*(23), 5410-5419.

Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLoS biology, 6*(8), e216.

Clawson, B. C., Durkin, J., & Aton, S. J. (2016). Form and function of sleep spindles across the lifespan. *Neural plasticity, 2016*.

Cooley, J. W., & Tukey, J. W. (1965). An algorithm for the machine calculation of complex Fourier series. *Mathematics of computation, 19*(90), 297-301.

Crais, E., McComish, C., Humphreys, E., Watson, L., Baranek, T., Reznick, J., Christian, R., & Earls, M. (2014). Pediatric healthcare professionals' views on autism spectrum disorder screening at 12–18 months. *Journal of Autism and Developmental Disorders*, 44, 2311–2328. doi: 10.1007/s10803-014-2101-2

Cremone, A., Kurdziel, L. B., Fraticelli-Torres, A., McDermott, J. M., & Spencer, R. (2017). Napping reduces emotional attention bias during early childhood. *Developmental science, 20*(4).

Cynader, M., & Mitchell, D. E. (1980). Prolonged sensitivity to monocular deprivation in dark-reared cats. *Journal of neurophysiology, 43*(4), 1026-1040.

Daoust, A.-M., Limoges, É., Bolduc, C., Mottron, L., & Godbout, R. (2004). EEG spectral analysis of wakefulness and REM sleep in high functioning autistic spectrum disorders. *Clinical neurophysiology, 115*(6), 1368-1373.

David, N., Schneider, T. R., Peiker, I., Al-Jawahiri, R., Engel, A. K., & Milne, E. (2016). Variability of cortical oscillation patterns: A possible endophenotype in autism spectrum disorders? *Neuroscience & Biobehavioral Reviews, 71*, 590-600.

D'Atri, A., Novelli, L., Ferrara, M., Bruni, O., & De Gennaro, L. (2018). Different maturational changes of fast and slow spindles in the first four years of life. *Sleep Medicine, 42*, 73-82.

Dawson, G., Jones, E. J., Merkle, K., Venema, K., Lowy, R., Faja, S., . . . Winter, J. (2012). Early behavioral intervention is associated with normalized brain activity in young children with autism. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(11), 1150-1159.

de Andrés, I., Garzón, M., & Reinoso-Suárez, F. (2011). Functional anatomy of non-REM sleep. *Frontiers in neurology, 2*.

De Gennaro, L., & Ferrara, M. (2003). Sleep spindles: an overview: Elsevier.

De Gennaro, L., Ferrara, M., Vecchio, F., Curcio, G., & Bertini, M. (2005). An electroencephalographic fingerprint of human sleep. *NeuroImage, 26*(1), 114-122.

Deoni, S. C., Dean, D. C., 3rd, Remer, J., Dirks, H., & O'Muircheartaigh, J. (2015). Cortical maturation and myelination in healthy toddlers and young children. *NeuroImage, 115*, 147-161. doi:10.1016/j.neuroimage.2015.04.058.

Doo, S., & Wing, Y. K. (2006). Sleep problems of children with pervasive developmental disorders: correlation with parental stress. *Developmental medicine and child neurology, 48*(8), 650-655.

Doucette, M. R., Kurth, S., Chevalier, N., Munakata, Y., & LeBourgeois, M. K. (2015). Topography of Slow Sigma Power during Sleep is Associated with Processing Speed in Preschool Children. *Brain Sci, 5*(4), 494-508. doi:10.3390/brainsci5040494

Ellingson, R. J. (1982). Development of sleep spindle bursts during the first year of life. *Sleep, 5*(1), 39-46.

Esser, S. K., Hill, S. L., & Tononi, G. (2007). Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. *Sleep, 30*(12), 1617.

Fattinger, S., Jenni, O. G., Schmitt, B., Achermann, P., & Huber, R. (2014). Overnight changes in the slope of sleep slow waves during infancy. *Sleep, 37*(2), 245-253. doi:10.5665/sleep.3390.

Feinberg, I., & Floyd, T. (1979). Systematic trends across the night in human sleep cycles. *Psychophysiology, 16*(3), 283-291.

Ferrarelli, F., Huber, R., Peterson, M. J., Massimini, M., Murphy, M., Riedner, B. A., . . . Tononi, G. (2007). Reduced sleep spindle activity in schizophrenia patients. *American Journal of Psychiatry, 164*(3), 483-492.

Filipek, P. A., Accardo, P. J., Baranek, G. T., Cook Jr, E. H., Dawson, G., Gordon, B., . . . Levy, S. E. (1999). The screening and diagnosis of autistic spectrum disorders. *Journal of autism and developmental disorders, 29*(6), 439-484.

Finelli, Achermann, P., & Borbély, A. (2001). Individual 'fingerprints' in human sleep EEG topography. *Neuropsychopharmacology, 25*(5), S57-S62.

Finelli, Baumann, H., Borbély, A., & Achermann, P. (2000). Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience, 101*(3), 523-529.

Fogel, Nader, Cote, & Smith. (2007). Sleep spindles and learning potential. *Behavioral neuroscience, 121*(1), 1.

Fox, S. E., Levitt, P., & Nelson, C. (2010). How the timing and quality of early experiences influence the development of brain architecture. *Child development, 81*(1), 28-40.

Fröhlich, F. (2016). *Network neuroscience*: Academic Press.

Frohlich J, Senturk D, Saravanapandian V, Golshani P, Reiter LT, Sankar R, et al. (2016) A quantitative electrophysiological biomarker of duplication 15q11.2-q13.1 syndrome. *PLoS ONE* 11(12): e0167179.doi:10.1371/journal. pone.0167179

Gabard-Durnam, L., Tierney, A. L., Vogel-Farley, V., Tager-Flusberg, H., & Nelson, C. A. (2015). Alpha asymmetry in infants at risk for autism spectrum disorders. *Journal of autism and developmental disorders*, *45*(2), 473-480.

Gardner, R. J., Kersanté, F., Jones, M. W., & Bartsch, U. (2014). Neural oscillations during non-rapid eye movement sleep as biomarkers of circuit dysfunction in schizophrenia. *European Journal of Neuroscience*, *39*(7), 1091-1106.

Gabsteiger, F., Leutheuser, H., Reis, P., Lochmann, M., & Eskofier, B.M. (2014) ICA-based reduction of electromyogenic artifacts in EEG data: Comparison with and without EMG data. Conference Proceeding Engineer Medical Biology Society. doi: 10.1109/EMBC.2014.6944466

Glannon, W. (2007). Neurodiversity. *Journal of Ethics in Mental Health.* (2), 1–6 .

Gaudreau, H., Carrier, J., & Montplaisir, J. (2001). Age-related modifications of NREM sleep EEG: from childhood to middle age. *Journal of sleep research*, *10*(3), 165-172.

Geiger, A, Huber, R, Kurth,S. Ringli, M., Jenni,O., & Achermann,P. (2011). The sleep EEG as a marker of intellectual ability in school age children. Sleep, (34), 2, 181–189.

Geyer, J. D., Talathi, S., & Carney, P. R. (2009). Introduction to sleep and polysomnography. *Clinical Sleep Disorders. Philadelphia: Lippincott Williams & Wilkins*, 265-266.

Ghuman, A. S., van den Honert, R. N., Huppert, T. J., Wallace, G. L., & Martin, A. (2017). Aberrant Oscillatory Synchrony Is Biased Toward Specific Frequencies and Processing Domains in the Autistic Brain. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *2*(3), 245-252.

Giannotti, F., Cortesi, F., Cerquiglini, A., Miraglia, D., Vagnoni, C., Sebastiani, T., & Bernabei, P. (2008). An investigation of sleep characteristics, EEG abnormalities and epilepsy in

developmentally regressed and non-regressed children with autism. *Journal of autism and developmental disorders, 38*(10), 1888-1897.

Giannotti, F., Cortesi, F., Cerquiglini, A., Vagnoni, C., & Valente, D. (2011). Sleep in children with autism with and without autistic regression. *Journal of sleep research, 20*(2), 338-347.

Gibbs, F. A., & Gibbs, E. L. (1941). Atlas of electroencephalography.

Gibbs, E.L., & Gibbs, F.A.(1962) Extreme spindles: correlation of electroencephalographic sleep pattern with mental retardation. *Science* 138(3545),1106–1110.

Gilmore, J. H., Shi, F., Woolson, S. L., Knickmeyer, R. C., Short, S. J., Lin, W., . . . Shen, D. (2012). Longitudinal development of cortical and subcortical gray matter from birth to 2 years. *Cereb Cortex, 22*(11), 2478-2485. doi:10.1093/cercor/bhr327.

Godbout, R., Bergeron, C., Limoges, E., Stip, E., & Mottron, L. (2000). A laboratory study of sleep in Asperger's syndrome. *Neuroreport, 11*(1), 127-130.

Gómez, R. L., Bootzin, R. R., & Nadel, L. (2006). Naps promote abstraction in language-learning infants. *Psychological science, 17*(8), 670-674.

Gray, P. H., Edwards, D.M., O'Callaghan, M.J., & Gibbons, K. (2015). Screening for autism spectrum disorder in very preterm infants during early childhood. *Early Human Development, 91*, 271 - 276.

Gruber, R., Wise, S., & Frenette (2013). The association between sleep spindles and IQ in healthy school-age children. *International Journal of Psychophysiology*, (89), 2, 229–240.

Gruber, R. & Wise, S. (2016). Sleep spindle characteristics in children with neurodevelopmental disorders and their relation to cognition. *Neural Plasticity,* 27 doi.10.1155/2016/4724792.

Guinchat, V., Thorsen, P., Laurant, C., Cans, C., & Cohen, D. (2012). Pre- and peri- and neonatal risk factors for autism. Obstectricia et Gynecologica Scandinavica, 91, 287-300.

Hoedlmoser, K., Heib, D., & Roelletal, J., (2014) Slow sleep spindle activity, declarative memory, and general cognitive abilities in children. *Sleep*. (37), 9, 1501–1512.

Hu, P., Stylos-Allan, M., & Walker, M. P. (2006). Sleep facilitates consolidation of emotional declarative memory. *Psychological science, 17*(10), 891-898.

Huber, & Born, J. (2014). Sleep, synaptic connectivity, and hippocampal memory during early development. *Trends Cogn Sciience. 18*(3), 141-152. doi:10.1016/j.tics.2013.12.005.

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature, 430*(6995), 78-81.

Huber, R., Ghilardi, M. F., Massimini, M., Ferrarelli, F., Riedner, B. A., Peterson, M. J., & Tononi, G. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nature Neuroscience, 9*(9), 1169-1176. doi:10.1038/nn1758.

Hupbach, A., Gomez, R. L., Bootzin, R. R., & Nadel, L. (2009). Nap-dependent learning in infants. *Developmental science, 12*(6), 1007-1012.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of comparative Neurology, 387*(2), 167-178.

Iber, C., Caples, S. M., Rosen, C. L., Shen, W. K., Gami, A. S., Cotts, W., Stepanski, E. (2007). The scoring of cardiac events during sleep. *J Clin Sleep Med, 3*(2), 147-154.

Jankel, W., & Niedermeyer, E. (1985). Sleep spindles. *Journal of clinical neurophysiology, 2*(1), 1-36.

Jenni, O. G., Borbely, A. A., & Achermann, P. (2004). Development of the nocturnal sleep electroencephalogram in human infants. *Am J Physiol Regul Integr Comp Physiol, 286*(3), R528-538. doi:10.1152/ajpregu.00503.2003.

Jeste, S. S., Frohlich, J., & Loo, S. K. (2015). Electrophysiological biomarkers of diagnosis and outcome in neurodevelopmental disorders. *Current opinion in neurology, 28*(2), 110-116.

Kandel, A., & Buzsáki, G. (1997). Cellular–synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *Journal of Neuroscience, 17*(17), 6783-6797.

Keenan, S., & Hirshkowitz, M. (2011). Monitoring and staging human sleep. *Principles and practice of sleep medicine, 5*, 1602-1609.

King, L. (1997). The history of ideas of autism. *Autism*,(1)1,13-23. doi.org/10.1177/1362361397011004.

King, K., Littlefield, A., McCabe, C., Mills, K., Flournoy, J., & Chassin, L. (2017). Longitudinal modeling in developmental neuroimaging research: Common challenges, and solutions from developmental psychology.*Developmental Cognitive Neuroscience*, doi.org/10.1016/j.dcn.2017.11.009.

Kotsopoulos, S. (2014). Early diagnosis of autism: Phenotype-endophenotype. *Psychiatrike= Psychiatriki, 25*(4), 273-281.

Kozhushko, N.J., Nagornova, Z.V., Evdokimov, S.A., Shemyakina, N.V., Ponomarev V.A., Tereshchenko, E.P., & Kropotov, J.D.(2018). Specificity of spontaneous EEG associated with different levels of cognitive and communicative dysfunctions in children. *International Journal of Psychophysiology*, 128, 22-30. doi: 10.1016/j.ijpsycho.2018.03.013.

Kuo, T., Chen, C.Y., Hsu, Y.C., & Yang, C. (2016). EEG beta power and heart rate variability describe the association between cortical and autonomic arousals across sleep. *Autonomic Neuroscience: Basic and Clinical*. 194. 32–37.

Kurdziel, L., Duclos, K., & Spencer, R. M. (2013). Sleep spindles in midday naps enhance learning in preschool children. *Proceedings of the National Academy of Sciences, 110*(43), 17267-17272.

Kurth. (2010). Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. *J Neurosci, 30*(40), 13211-13219. doi:10.1523/JNEUROSCI.2532-10.2010.

Kurth, Achermann, P., Rusterholz, T., & Lebourgeois, M. K. (2013). Development of Brain EEG Connectivity across Early Childhood: Does Sleep Play a Role? *Brain Sci, 3*(4), 1445-1460. doi:10.3390/brainsci3041445.

Kurth, S., Dean III, D. C., Achermann, P., O'Muircheartaigh, J., Huber, R., Deoni, S. C., & LeBourgeois, M. K. (2016). Increased sleep depth in developing neural networks: new insights from sleep restriction in children. *Frontiers in human neuroscience, 10*.

Kurth, Jenni, O. G., Riedner, B. A., Tononi, G., Carskadon, M. A., & Huber, R. (2010). Characteristics of sleep slow waves in children and adolescents. *Sleep, 33*(4), 475-480.

Kurth, Ringli, M., LeBourgeois, M. K., Geiger, A., Buchmann, A., Jenni, O. G., & Huber, R. (2012). Mapping the electrophysiological marker of sleep depth reveals skill maturation in children and adolescents. *NeuroImage, 63*(2), 959-965.

Larsson, H.J., Eaton, W.W., Madsen, K.M., Vestergaard, M., Olseon, A.V., & Argerbo, E. (2005). Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. *Am Journal of Epidemiology, 161*, 916 – 925.

Lee, G. M., Fattinger, S., Mouthon, A. L., Noirhomme, Q., & Huber, R. (2013). Electroencephalogram approximate entropy influenced by both age and sleep. *Front Neuroinform, 7*, 33. doi:10.3389/fninf.2013.00033.

Léveillé, C., Barbeau, E. B., Bolduc, C., Limoges, É., Berthiaume, C., Chevrier, É., . . . Godbout, R. (2010). Enhanced connectivity between visual cortex and other regions of the brain in autism: a REM sleep EEG coherence study. *Autism Research, 3*(5), 280-285.

Levin, A. R., Zeanah, C. H., Jr., Fox, N. A., & Nelson, C. A. (2014). Motor outcomes in children exposed to early psychosocial deprivation. *J Pediatr, 164*(1), 123-129 e121. doi:10.1016/j.jpeds.2013.09.026.

Lifter, K., & Bloom, L. (1989). Object knowledge and the emergence of language. *Infant Behavior and Development, 12*(4), 395-423.

Limoges, Mottron, Bolduc, Berthiaume, & Godbout. (2005). Atypical sleep architecture and the autism phenotype. *Brain, 128*(5), 1049-1061.

Liptak, G. S., Benzoni, L. B., Mruzek, D. W., Nolan, K. W., Thingvoll, M. A., Wade, C. M., & Fryer, G. E. (2008). Disparities in diagnosis and access to health services for children with autism: data from the National Survey of Children's Health. *J Dev Behav Pediatr, 29*(3), 152-160. doi:10.1097/DBP.0b013e318165c7a0.

Luckhardt, C., Jarczok, T., & Bender, S. (2014). Elucidating the neurophysiological underpinnings of autism spectrum disorder: new developments. *Journal of Neural Transmission, 121*(9), 1129-1144.

Lustenberger, C., Maric, A., Durr, R., Achermann, P., & Huber, R. (2012). Triangular relationship between sleep spindle activity, general cognitive ability and the efficiency of declarative learning. *PLoS One, 7*(11), e49561. doi:10.1371/journal.pone.0049561.

Lustenberger, C., Mouthon, A. L., Tesler, N., Kurth, S., Ringli, M., Buchmann, A., . . . Huber, R. (2017). Developmental trajectories of EEG sleep slow wave activity as a marker for motor skill development during adolescence: a pilot study. *Dev Psychobiol, 59*(1), 5-14. doi:10.1002/dev.21446.

Lustenberger, C., Murbach, M., Tushaus, L., Wehrle, F., Kuster, N., Achermann, P., & Huber, R. (2015). Inter-individual and intra-individual variation of the effects of pulsed RF EMF exposure on the human sleep EEG. *Bioelectromagnetics, 36*(3), 169-177. doi:10.1002/bem.21893.

Lustenberger, C., O'Gorman, R. L., Pugin, F., Tushaus, L., Wehrle, F., Achermann, P., & Huber, R. (2015). Sleep spindles are related to schizotypal personality traits and thalamic glutamine/glutamate in healthy subjects. *Schizophr Bull, 41*(2), 522-531. doi:10.1093/schbul/sbu109.

Lustenberger, C., Wehrle, F., Tushaus, L., Achermann, P., & Huber, R. (2015). The Multidimensional Aspects of Sleep Spindles and Their Relationship to Word-Pair Memory Consolidation. *Sleep, 38*(7), 1093-1103. doi:10.5665/sleep.4820

Lüthi, A. (2014). Sleep spindles: where they come from, what they do. *The Neuroscientist, 20*(3), 243-256.

Luyster, R. J., Wagner, J. B., Vogel-Farley, V., Tager-Flusberg, H., & Nelson, C. A, (2011). Neural correlates of familiar and unfamiliar face processing in infants at risk for autism spectrum disorders. *Brain Topography, 24*(3), 220–228.

Malow, B. A., Marzec, M. L., McGrew, S. G., Wang, L., Henderson, L. M., & Stone, W. L. (2006a). Characterizing sleep in children with autism spectrum disorders: a multidimensional approach. *Sleep, 29*(12), 1563-1571.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444*(7119), 610.

Massimini, M., Tononi, G., & Huber, R. (2009). Slow waves, synaptic plasticity and information processing: insights from transcranial magnetic stimulation and high-density EEG experiments. *European Journal of Neuroscience, 29*(9), 1761-1770.

Maxwell, C. R., Villalobos, M. E., Schultz, R. T., Herpertz-Dahlmann, B., Konrad, K., & Kohls, G. (2015). Atypical laterality of resting gamma oscillations in autism spectrum disorders. *Journal of autism and developmental disorders, 45*(2), 292-297.

McClain, I. J., Lustenberger, C., Achermann, P., Lassonde, J. M., Kurth, S., & LeBourgeois, M. K. (2016). Developmental Changes in Sleep Spindle Characteristics and Sigma Power across Early Childhood. *Neural Plast, 2016*, 3670951. doi:10.1155/2016/3670951

McDonald, J.H. 2014. Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland.

McPartland, J. C. (2016). Considerations in biomarker development for neurodevelopmental disorders. *Current opinion in neurology, 29*(2), 118-122.

Mullen, E. M. (1995). Mullen Scales of Early Learning (AGS ed.). Circle Pines, MN: American Guidance Service Inc.

Murphy, M., Riedner, B. A., Huber, R., Massimini, M., Ferrarelli, F., & Tononi, G. (2009). Source modeling sleep slow waves. *Proceedings of the National Academy of Sciences, 106*(5), 1608-1613.

Newport, E. L., Bavelier, D., & Neville, H. J. (2001). Critical thinking about critical periods: Perspectives on a critical period for language acquisition. *Language, brain and cognitive development: Essays in honor of Jacques Mehler*, 481-502.

Nichols, T. E., & Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Human brain mapping, 15*(1), 1-25.

Novelli, L., D'Atri, A., Marzano, C., Finotti, E., Ferrara, M., Bruni, O., & De Gennaro, L. (2016). Mapping changes in cortical activity during sleep in the first 4 years of life. *J Sleep Res, 25*(4), 381-389. doi:10.1111/jsr.12390.

Nunez, P. L., & Srinivasan, R. (2006). *Electric fields of the brain: the neurophysics of EEG*: Oxford University Press, USA.

Olbrich, E., Rusterholz, T., LeBourgeois, M., & Achermann, P. (2017). Developmental changes in sleep oscillations during early childhood. *Neural Plasticity*. doi.org/10.1155/2017/6160959.

Ornitz, E. M., Ritvo, E. R., Brown, M. B., La Franchi, S., Parmelee, T., & Walter, R. D. (1969). The EEG and rapid eye movements during REM sleep in normal and autistic children. *Electroencephalography and clinical neurophysiology, 26*(2), 167-175.

Ozonoff, S., Baguio, F., Cook, I. C., Hill, M. M., Hutman, T., & Sigman, M. (2010). A prospective study of the emergence of early behavioral signs of autism. *Journal of the American Academy of Child & Adolescent Psychiatry, 49*(3), 256-266. e252.

Ozonoff, Young, G. S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., . . . Stone, W. L. (2011). Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. *Pediatrics, 128*(3), e488-495. doi:10.1542/peds.2010-2825

Ozonoff, Young, G. S., Landa, R. J., Brian, J., Bryson, S., Charman, T., . . . Stone, W. L. (2015). Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *Journal of Child Psychology and Psychiatry, 56*(9), 988-998.

Page, J., Lustenberger, C., & Frohlich, F. (2018). Social, motor, and cognitive development through the lens of sleep network dynamics in infants and toddlers between 12 and 30 months of age. SLEEP, 41 (4) https://doi.org/10.1093/sleep/zsy024

Payne, J. D., Stickgold, R., Swanberg, K., & Kensinger, E. A. (2008). Sleep preferentially enhances memory for emotional components of scenes. *Psychological science, 19*(8), 781-788.

Plante, D. T., Goldstein, M. R., Cook, J. D., Smith, R., Riedner, B. A., Rumble, M. E., . . . Peterson, M. J. (2016). Effects of partial sleep deprivation on slow waves during non-rapid eye movement sleep: A high density EEG investigation. *Clin Neurophysiol, 127*(2), 1436-1444. doi:10.1016/j.clinph.2015.10.040

Polimeni, M., Richdale, A., & Francis, A. (2005). A survey of sleep problems in autism, Asperger's disorder and typically developing children. *Journal of Intellectual Disability Research, 49*(4), 260-268.

Polleux, F., & Lauder, J. M. (2004). Toward a developmental neurobiology of autism. *Developmental Disabilities Research Reviews, 10*(4), 303-317.

Pop-Jordanova N, Zorcec T, Demerdzieva A, Gucev Z. (2010). QEEG characteristics and spectrum weighted frequency for children diagnosed as autistic spectrum disorder. *Nonlinear Biomedical Physics*.

Rasch, B., & Born, J. (2013). About Sleeps Role in Memory. *Physiological reviews, 93*(2), 681-766.

Rechtschaffen, A., & Kales, A. (1968). A manual of standardized terminology, techniques, and scoring systems for sleep stages of human subjects.

Redcay, E., & Courchesne, E. (2005). When is the brain enlarged in Autism? A meta-analysis of all brain size reports. Biological Psychiatry, 58, 1–9.

Richdale, A. L., & Schreck, K. A. (2009). Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep medicine reviews, 13*(6), 403-411.

Righi, G., Tierney, A. L., Tager-Flusberg, H., & Nelson, C. A. (2014). Functional connectivity in the first year of life in infants at risk for autism spectrum disorder: an EEG study. *PLoS One, 9*(8), e105176.

Rihm, J. S., Diekelmann, S., Born, J., & Rasch, B. (2014). Reactivating memories during sleep by odors: odor specificity and associated changes in sleep oscillations. *Journal of cognitive neuroscience, 26*(8), 1806-1818.

Ringli, M., & Huber, R. (2011). 5 Developmental aspects of sleep slow waves: Linking sleep, brain maturation and behavior. *Progress in brain research, 193*, 63.

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O. G., & Huber, R. (2013). Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder. *Cortex, 49*(1), 340-347.

Rochette, A., Soulieres, I., Berthiaume, C., & Godbout, R. (2018). NREM sleep EEG activity and procedural memory: A comparison between young neurotypical and autistic adults without sleep complaints. Autism Research. 00: 00–00. doi: 10.1002/aur.1933

Rojas, D. C., & Wilson, L. B. (2014). γ-band abnormalities as markers of autism spectrum disorders. *Biomarkers in medicine, 8*(3), 353-368.

Ruggeri, B., Sarkans, U., Schumann, G., & Persico, A. M. (2014). Biomarkers in autism spectrum disorder: the old and the new. *Psychopharmacology, 231*(6), 1201-1216.

Sankupellay, M., Wilson, S., Heussler, H. S., Parsley, C., Yuill, M., & Dakin, C. (2011). Characteristics of sleep EEG power spectra in healthy infants in the first two years of life. *Clin Neurophysiol, 122*(2), 236-243. doi:10.1016/j.clinph.2010.06.030.

Sasso, G., Bat-Pitault., F., Guyon, A., Plancoulaine, S., Banfi, T., Milioli, B., Parrino, L., Faraguna, U., & Franco, P. (2017). Electrophysiological and microstructural features of sleep in children at high risk for depression a preliminary study. *Sleep Medicine*. (36), 95-113. doi:10.1016/j05.013 1389-9457

Schabus, M., Hödlmoser, K., Gruber, G., Sauter, C., Anderer, P., Klösch, G., . . . Zeitlhofer, J. (2006). Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. *European Journal of Neuroscience, 23*(7), 1738-1746.

Scherf, K. S., Behrmann, M., Humphreys, K., & Luna, B. (2007). Visual category-selectivity for faces, places and objects emerges along different developmental trajectories. *Developmental science, 10*(4).

Scholle, S., Zwacka, G., & Scholle, H. (2007). Sleep spindle evolution from infancy to adolescence. *Clinical neurophysiology, 118*(7), 1525-1531.

Schumacher, A. M., Miller, A. L., Watamura, S. E., Kurth, S., Lassonde, J. M., & LeBourgeois, M. K. (2017). Sleep moderates the association between response inhibition and self-regulation in early childhood. *Journal of Clinical Child & Adolescent Psychology, 46*(2), 222-235.

Shaw, P., Eckstrand, K., Sharp, W., Blumenthal, J., Lerch, J., Greenstein, D., . . . Rapoport, J. (2007). Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proceedings of the National Academy of Sciences, 104*(49), 19649-19654.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., . . . Rapoport, J. L. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *Journal of Neuroscience, 28*(14), 3586-3594.

Shinomiya, S., Nagata, K., Takahashi, K., & Masumura, T. (1999). Development of sleep spindles in young children and adolescents. *Clinical Electroencephalography, 30*(2), 39-43.

Shonkoff, J. P. (2000). In J. P. Shonkoff & D. A. Phillips (Eds.), *From Neurons to Neighborhoods: The Science of Early Childhood Development*. Washington (DC).

Simon, D. M., & Wallace, M. T. (2016). Dysfunction of sensory oscillations in autism spectrum disorder. *Neuroscience & Biobehavioral Reviews, 68*, 848-861.

Singh, V. K., Lin, S. X., Newell, E., & Nelson, C. (2002). Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. *Journal of biomedical science, 9*(4), 359-364.

Sparrow, S., Cicchetti, D. & Balla, A. 2005. Vineland adaptive behavior scales, Circle Pines, MN, American Guidance Service.

Srinivasan, R., Nunez, P. L., & Silberstein, R. B. (1998). Spatial filtering and neocortical dynamics: estimates of EEG coherence. *IEEE Transactions on Biomedical Engineering, 45*(7), 814-826.

Srinivasan, R., Winter, W. R., & Nunez, P. L. (2006). Source analysis of EEG oscillations using high-resolution EEG and MEG. *Progress in brain research, 159*, 29-42.

Steriade, M. (2006). Grouping of brain rhythms in corticothalamic systems. *Neuroscience, 137*(4), 1087-1106.

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature, 437*(7063), 1272.

Stroganova, T. A., Orekhova, E. V., & Posikera, I. N. (1999). EEG alpha rhythm in infants. *Clinical neurophysiology, 110*(6), 997-1012.

Strzelecka, J. (2014). Electroencephalographic studies in children with autism spectrum disorders. *Research in Autism Spectrum Disorders, 8*(3), 317-323.

Tager-Flusberg, H., & Joseph, R. M. (2003). Identifying neurocognitive phenotypes in autism. *Philos Trans R Soc Lond B Biol Sci, 358*(1430), 303-314. doi:10.1098/rstb.2002.1198.

Tanguay, P. E., Ornitz, E. M., Forsythe, A. B., & Ritvo, E. R. (1976). Rapid eye movement (REM) activity in normal and autistic children during REM sleep. *Journal of autism and developmental disorders, 6*(3), 275-288.

Tanguay, P. E., Ornitz, E. M., Kaplan, A., & Bozzo, E. S. (1975). Evolution of sleep spindles in childhood. *Electroencephalography and clinical neurophysiology, 38*(2), 175-181.

Tarokh, L., Carskadon, M., & Achermann,P (2010). Developmental changes in brain connectivity assessed using the sleep EEG. *Neuroscience*, 171(2), 622–634.

Taylor, M. A., Schreck, K. A., & Mulick, J. A. (2012). Sleep disruption as a correlate to cognitive and adaptive behavior problems in autism spectrum disorders. *Research in developmental disabilities, 33*(5), 1408-1417.

Tessier, & et al. (2015). Intelligence measures and stage 2 sleep in typically-developing and autistic children. *International Journal of Psychophysiology, 97*(1), 58-65.

Tessier, S., Lambert, A., Scherzer, P., Jemel, B., & Godbout, R. (2015). REM sleep and emotional face memory in typically-developing children and children with autism. *Biological psychology, 110*, 107-114.

Tierney,S., Gabard-Durnam, L., Vogel-Farley, V., Tager-Flusberg, H., & Nelson, C. A. (2012). Developmental trajectories of resting EEG power: an endophenotype of autism spectrum disorder. *PLoS One, 7*(6), e39127.

Tierney,S. & Nelson, C. A. (2009). Brain Development and the Role of Experience in the Early Years. *Zero Three, 30*(2), 9-13.

Timofeev, I., Bazhenov, M., Seigneur, J., & Sejnowski, T. (2012). Neuronal synchronization and thalamocortical rhythms in sleep, wake and epilepsy.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep medicine reviews, 10*(1), 49-62.

Trevaud, K., Ure, A., Doyle, L.W., Lee, K.J., Rogers, C.E, & Kidokoro, H. (2013). Psychiatric outcomes at age seven for very preterm children: rates and predictors. Journal of Psychological Psychiatry, 54, 772 - 779.

United States. Individuals with Disabilities Education Improvement Act of 2004. [Bethesda, MD :ProQuest].

Varcin, K. J., & Nelson, C. A. (2016). A developmental neuroscience approach to the search for biomarkers in autism spectrum disorder. *Current opinion in neurology, 29*(2), 123-129.

Ventola, P., Kleinman, J., Pandey, J., Wilson, L., Esser, E., Boorstein, H., Fein, D. (2007). Differentiating between autism spectrum disorders and other developmental disabilities in children who failed a screening instrument for ASD. *J Autism Dev Disord, 37*(3), 425-436. doi:10.1007/s10803-006-0177-z.

Vyazovskiy, V. V., Olcese, U., Lazimy, Y. M., Faraguna, U., Esser, S. K., Williams, J. C., Tononi, G. (2009). Cortical firing and sleep homeostasis. *Neuron, 63*(6), 865-878.

Walsh, P., Elsabbagh, M., Bolton, P., & Singh, I. (2011). In search of biomarkers for autism: scientific, social and ethical challenges. *Nature Reviews Neuroscience, 12*(10), 603-612.

Wang, J., Barstein, J., Ethridge, L. E., Mosconi, M. W., Takarae, Y., & Sweeney, J. A. (2013). Resting state EEG abnormalities in autism spectrum disorders. *Journal of neurodevelopmental disorders, 5*(1), 24.

Webb, S.J., Bernier, R., Henderson, H.A., Johnson, M.A, Jones, E.J., Lerner, M.D., McPartland, J.C., Nelson, C.A., Rojas, D.C., Townsend, J., & Westerfield, M. (2015) Guidelines and best practices for electrophysiological data collection, analysis and reporting in autism. *Journal Autism Developmental Disorders*, 45,425–443. doi: 10.1007/s10803-013-1916-6.

Webb, S.J., Jones E. J. H, Merkle, K., Murias, M., Greenson, J., Richards, T., Aylward, E., & Dawson G. (2010) Response to familiar faces, newly familiar faces, and novel faces as assessed by ERPs is intact in adults with autism spectrum disorders. *International Journal of Psychophysiology, 77(2),* 106–117.

Webb, S. J., Jones, E. J. H., Merkle, K., Venema, K., Greenson, J., Murias, M., & Dawson, G. (2011). Developmental change in the ERP responses to familiar faces in toddlers with autism spectrum disorders versus typical development. *Child Development, 82*(6), 1868-1886. doi:10.1111/j.1467-8624.2011.01656.x

Webb, S. J., Merkle, K., Murias, M., Richards, T., Aylward, E., & Dawson, G. (2012). ERP responses differentiate inverted but not upright face processing in adults with ASD. *Social Cognitive and Affective Neuroscience,* 7, 578–587

Wilhelm, I., Kurth, S., Ringli, M., Mouthon, A.-L., Buchmann, A., Geiger, A., . . . Huber, R. (2014). Sleep slow-wave activity reveals developmental changes in experience-dependent plasticity. *Journal of Neuroscience, 34*(37), 12568-12575.

Yasuhara, A. (2010). Correlation between EEG abnormalities and symptoms of autism spectrum disorder (ASD). *Brain and Development, 32*(10), 791-798.

Zhao, X., Modur, V., Carayannopoulos, L. N., & Laterza, O. F. (2015). Biomarkers in pharmaceutical research. *Clinical chemistry, 61*(11), 1343-1353.

Zwaigenbaum L., Bauman M.L., Fein D., Pierce K., Buie T., Davis P.A., Newschaffer C., Robins D.L., Wetherby A., Choueiri R., Kasari C., Stone W.L., Yirmiya N., Estes A., Hansen R.L., McPartland J.C., Natowicz M.R., Carter A., Granpeesheh, D., Mailloux Z., Smith Roley S., & Wagner S. (2015). Early screening of autism spectrum disorder: Recommendations for practice and research. *Pediatrics, 136(1*), 41-59. doi: 10.1542.